CHAPTER 2: OVERVIEW OF WARFARIN AND DRUG-DRUG INTERACTIONS

2.1 INTRODUCTION

This chapter consists of a brief look into the history of warfarin, how it is pharmacologically classified and what it is indicated for. Attention will also be given to the pharmacokinetics and pharmacodynamics of warfarin. Other topics that will be discussed are possible interactions. A brief summary on the types of interactions will also be provided.

2.2 THE BACKGROUND OF WARFARIN

This section focuses on how warfarin came to be in clinical practice, its pharmacological classification and its indications.

2.2.1 HISTORY

Since the times of Hippocrates, leeches have been used for bloodletting. Today, leeches are still being used medicinally for the purpose of thrombosis prevention. Surgeons use the leech *Hirudo medicinalis* in the prevention of thrombosis in the fine blood vessels of digits that have been reattached (Katzung *et al.*, 2009:593). Today, however, there are a range of anticoagulants that can be used for this purpose. One such anticoagulant is warfarin.

Anticoagulants were discovered in the 1920s when it was found that cattle suffered from haemorrhagic disorders caused by the ingestion of sweet clover silage that have spoiled (Fitzpatrick & O'Kennedy, 2004:11). It was later discovered that the compound causing these haemorrhagic disorders is bishydroxycoumarin or dicoumarol (Brunton, 2012; Fitzpatrick & O'Kennedy, 2004:11). Dicoumarol is formed in spoiled sweet clover hay when coumarin is oxidised to 4-hydroxycoumarin. When this reacts with formaldehyde it leads to the formation of dicoumarol (Fitzpatrick & O'Kennedy, 2004:11).

In 1948 Link and his colleagues discovered the compound warfarin while synthesising over a 100 types of 4-hydroxycourmarin compounds. They found that an intact 4-hydroxycoumarin residue where the 3-position is substituted by a carbon is the structural requirement for anticoagulant activity (Brunton, 2012; Fitzpatrick & O'Kennedy, 2004:12). This discovery was made at the University of Wisconsin. The name warfarin is derived from the name of the Wisconsin Alumni Research Foundation with the added "arin" from coumarin (Katzung *et al.*,

2009:594). Warfarin was first used in the 1950s and has since then become the most frequently prescribed anticoagulant for the management and treatment of thrombo-embolic disorders (Brunton, 2012).

2.2.2 PHARMACOLOGICAL CLASSIFICATION

Warfarin is mainly classified as a vitamin K antagonist (Brunton, 2012; D'Andrea *et al.*, 2008:127; Autar, 2009:165; Appadu, 2010:247). It is classified in this way because it interferes with the formation of the reduced form of vitamin K from its oxidised form (Hirsh *et al.*, 2003:S1633). Warfarin's complete mechanism of action will be discussed in a later section.

Warfarin is also known as a coumarin derivative (Hirsh *et al.*, 2003:1633). The chemical structures of these compounds consist of an aromatic ring attached to a condensed lactone ring (D'Andrea *et al.*, 2008:127). Other compounds in this group include phenprocoumon and acenocoumarol. These drugs are not as frequently prescribed as warfarin. Indandione derivatives such as anisindione and phenindione are similar to warfarin, but are not widely used due to their toxicity profile. Bromadiolone, brodifacoum, diphenadione, chlorophacinone and pindone are also part of these two groups, but are used as rodenticides (Brunton, 2012).

2.2.3 INDICATION

Warfarin is used in a wide range of thrombo-embolic disorders (Fitzpatrick & O'Kennedy, 2004:11). The following is a list of just a few of these conditions:

- The prevention and treatment of thrombo-embolic disorders (D'Andrea *et al.*, 2008:127).
- The treatment of venous thrombo-embolism and deep vein thrombosis (Autar, 2009:165, 166).
- The treatment and prevention of pulmonary embolism.
- The treatment of atrial fibrillation with a risk of embolisation.
- To prevent the deposition of thrombi on prosthetic heart valves (Appadu, 2010:252).
- The prevention of acute myocardial infarction in patients with peripheral arterial disease and in men generally at risk.
- The prevention of stroke.
- The prevention of recurrent infarction.
- The prevention of death in patients with acute myocardial infarction (Hirsh *et al.*, 2003:1642).

2.2.4 PHARMACOKINETICS

2.2.4.1 Chemistry and stability

The chemical name for warfarin is 4-hydroxy-3-(3-oxo-1-phenylbutyl) coumarin as it is a synthetic 3-substituted derivative of 4-hydroxycoumarin (AHFS, 2011; Sweetman, 2011). Warfarin is a white or almost white, hygroscopic, amorphous powder that has a bitter taste and that is discoloured when it comes into contact with light (AHFS, 2011; BP, 2011; Sweetman, 2011). It is very soluble in water and alcohol, soluble in acetone and very slightly soluble in chloroform, ether and dichloromethane (AHFS, 2011; Sweetman, 2011). Preparations usually contain warfarin sodium or warfarin sodium clathrate (BP, 2011). To eliminate the trace impurities that are normally present in amorphous warfarin, crystalline warfarin sodium is prepared from warfarin or amorphous warfarin. Crystalline warfarin sodium is an isopropanol clathrate compound (AHFS, 2011).

Table 2.1 is a summary of the incompatibilities of warfarin with other compounds as adapted from Sweetman (2011).

Table 2.1: Compounds incompatible with solutions of warfarin sodium

	INCOMPATIBLE COMPOUNDS
Solutions of warfarin sodium:	Adrenaline hydrochloride Amikacin sulphatesulphate Metaraminol tartrate Oxytocin Promazine hydrochloride Tetracycline hydrochloride
Visual incompatibility with solutions of warfarin sodium mixed with solutions of:	Aminophylline Bretylium tosilate Ceftazidime Cimetidine hydrochloride Ciprofloxacin lactate Dobutamine hydrochloride Esmolol hydrochloride Gentamicin sulphatesulphate Labetalol hydrochloride Metronidazole hydrochloride Vancomycin hydrochloride
Haze reported after 24 hours with:	Sodium chloride 0.9%

Warfarin sodium tablets should be stored in containers that are tight and light resistant as it discolours when exposed to light. Warfarin sodium for injection should be kept in its original carton until it is ready to be used. Lyophilised warfarin sodium for injection should be reconstituted with sterile water for injection. After this is done the solution containing 2mg per ml of warfarin sodium will have a pH between 8.1 and 8.3. This solution is stable for four hours at room temperature and it should not be refrigerated. If there is any reconstituted solution left, it should be discarded after four hours (AHFS, 2011).

2.2.4.2 Absorption

In clinical practice warfarin is administered as a racemic mixture of 2 optically active isomers, the *R*-enantiomer and the *S*-enantiomer (Vaz-Da-Silva *et al.*, 2010:180; Hirsh *et al.*, 2003:1633). The *S*-enantiomer's anticoagulant activity is up to 5 times more potent than that of the *R*-enantiomer (Fitzpatrick & O'Kennedy, 2004:12).

Absorption of warfarin from the gastro-intestinal tract is fast, but it varies considerably from person to person (AHFS, 2011; Appadu, 2010:252; Jay & Lui, 2006:36). The absorption of warfarin sodium is controlled by its dissolution rate and the absorption of the drug from oral tablets may differ from one brand to the other. The rate of absorption of the drug from the gastro-intestinal tract is affected by the presence of food in the gastro-intestinal tract. Recurring contact of warfarin with the skin can result in warfarin toxicity as warfarin can be absorbed percutaneously. This usually occurs with the repeated handling of rodenticides containing warfarin (AHFS, 2011).

After absorption from the gastro-intestinal tract warfarin binds to the plasma protein, albumin. Warfarin is approximately 97% bound to albumin and only the unbound drug ("free drug") is pharmacologically active (Appadu, 2010:252; D'Andrea *et al.*, 2008:128). Warfarin bound to albumin has a half-life of 36 to 42 hours (D'Andrea *et al.*, 2008:128). Peak plasma concentration is reached 60 to 90 minutes after oral administration of warfarin, although it can already be detected in the blood one hour after oral administration (D'Andrea *et al.*, 2008:128; Fitzpatrick & O'Kennedy, 2004:12). Plasma concentrations of warfarin do not correlate with its anticoagulant or antithrombogenic effect, so it is not an accurate way of determining the required dose for these effects. The peak plasma concentration is achieved faster with intravenous warfarin administration, but the anticoagulant or antithrombogenic effect is not augmented by this route of administration (AHFS, 2011). The most favourable therapeutic effect of warfarin is achieved three to five days after therapy has begun (Denooz *et al.*, 2009:2344). There are still active coagulation factors circulating in the blood on which warfarin has no effect. For antithrombogenic effects to occur, six days of warfarin therapy is needed. For the antithrombogenic effects to occur, a decrease in coagulation factor II

(prothrombin) is needed, and this has a half-life of approximately 60 to 72 hours. For anticoagulant effects to occur, two days of warfarin therapy is needed because a decrease in other coagulation factors is needed, which have half-lives of six to 24 hours (Hirsh *et al.*, 2003:1636).

After discontinuation of warfarin therapy it may take a while for normal blood coagulation to occur because plasma concentration levels of coagulation factors must return to normal (AHFS, 2011). This latency period is determined by the half-life of warfarin e.g. the longer the half-life the longer the latency period (Denooz *et al.*, 2009:2344). The onset of the effects of warfarin does not differ between the different roots of administration (orally, IM, IV). Larger doses of warfarin (doses beyond what is needed to affect the synthesis of coagulation factors IX and X) will not shorten the onset time of action, but will lengthen the latency period after warfarin treatment has been discontinued (AHFS, 2011).

2.2.4.3 Distribution

As mentioned before, warfarin is approximately 97% bound to the plasma protein, albumin (Appadu, 2010:252). It is distributed to various parts of the body. The most obvious place of distribution of warfarin is to the liver where it metabolised by the CYP450 enzyme system (Denooz *et al.*, 2009:2344). Other organs in the body where warfarin is distributed include the lungs, spleen and kidneys (AHFS: 2011). In pregnant women, warfarin can cross the placenta and can have teratogenic effects on the foetus in the first trimester of pregnancy (AHFS, 2011; Hirsh *et al.*, 2003:1641). The drug plasma concentrations of the foetus and the mother may be equivalent to each other (AHFS, 2011). Some sources state that warfarin is not distributed to breast milk (Brunton, 2012). Other sources are still sceptical, but agree that warfarin is not found in breast milk or the blood plasma of infants who are breast fed. The distribution of *R*-warfarin and *S*-warfarin are the same AHFS, 2011).

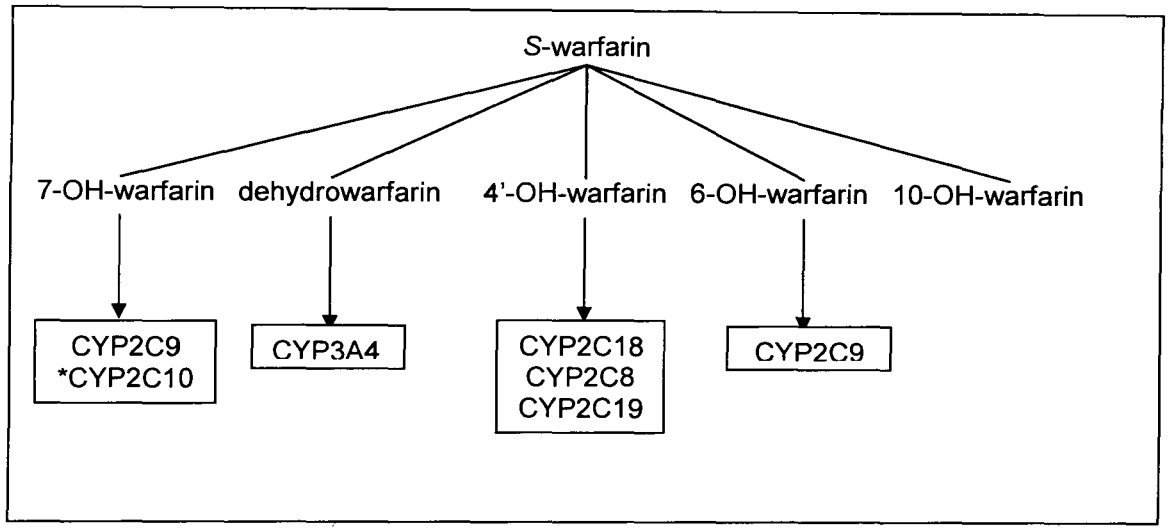
2.2.4.4 Metabolism

The chemical structure of warfarin contains an asymmetric carbon at position 9. This asymmetric carbon produces the two enantiomers of warfarin, namely *R*-warfarin and *S*-warfarin (Kaminsky & Zhang, 1997:67). Warfarin is clinically administered as a racemate of these two isomers or enantiomers (AHFS, 2011; Breccia *et al.*, 2010:e224; Miller *et al.*, 2008:2211). Warfarin mainly undergoes phase I metabolism. However, some of these metabolites also undergo phase II metabolism afterwards, resulting in metabolites that are glucuronidated and sulphated (Miller *et al.*, 2008:2211; Guo *et al.*, 2006). The metabolism of warfarin occurs when it binds to a chiral macromolecular complex (such as an enzyme), thus making its metabolism mainly stereoselective (Park, 1988). The enantiomers of warfarin are

metabolised by a variety of cytochrome P450 (CYP450) enzymes via different pathways (Anon., 2011; Guo *et al.*, 2006; Kaminsky & Zhang, 1997:67; Park, 1988). The metabolism by CYP450 produces multiple monohydroxylated metabolites. The metabolism of these enantiomers by the different forms of P450 enzymes occurs on different sites of the structure of the enantiomers. This process is described as the regioselective metabolism of warfarin. In other words, CYP1A2 primarily metabolises *R*-warfarin to produce 6-hydroxywarfarin and 8-hydroxywarfarin and therefore, CYP1A2 is stereoselective for *R*-warfarin and regioselective for 6-hydroxywarfarin and 8-hydroxywarfarin (Kaminsky & Zhang, 1997:67). The potencies of these two enantiomers differ as well (Kaminsky & Zhang, 1997:67; Park, 1988). The principle CYP450 enzyme responsible for the metabolism of warfarin is CYP2C9. The CYP2C9 enzyme hydroxylates warfarin to inactive metabolites (Moridani *et al.*, 2006:606; Stromich *et al.*, 2010:1931). The primary metabolites formed by this reaction are 6-, 7-, and 8-hydroxywarfarin (Park, 1988). As mentioned above (paragraph 2.2.4.4.), *R*-warfarin and *S*-warfarin are metabolised via different pathways, thus the metabolism of these two enantiomers will be discussed separately.

- ***The metabolism of S-warfarin***

The anticoagulant effect of *S*-warfarin is more potent than that of *R*-warfarin. *S*-warfarin is three to five times more potent than *R*-warfarin, but has a shorter half-life (Anon., 2011; Breccia *et al.*, 2010:e224; Moridani *et al.,* 2006:606). The primary enzyme responsible for the metabolism of *S*-warfarin is the CYP2C9 enzyme. The CYP2C9 enzyme transforms *S*-warfarin to 7-hydroxywarfarin. The 7-hydroxywarfarin metabolite is inactive and it is secreted in the bile (AHFS, 2011; Miller *et al.,* 2008:2211; Suzuki *et al.,* 2008:1155; Zuo *et al.*, 2010:305). Other CYP450 enzymes that are to a lesser extent responsible for the metabolism of warfarin are CYP2C8, CYP2C18, CYP3A4, CYP2C19, and to a much lesser extent CYP2C10 (AHFS, 2011; Kaminsky & Zhang, 1997:68; Yamazaki & Shimada 1997:1197). The major site of these enzymes is the liver, although they can be found extrahepatically as well (Yamazaki & Shimada, 1997:1195). Various metabolites are produced by these enzymes. In total, five metabolites are produced by the metabolism of *S*-warfarin namely, 7-hydroxywarfarin (which is primarily formed), dehydrowarfarin and 4'-, 6-, and to a lesser extent, 10-hydroxywarfarin (Anon., 2011; Kaminsky & Zhang 1997:68; Zhang *et al.*, 1997:390). Figure 2.1 is a summary of all the metabolites formed from the metabolism of *S*-warfarin and the various enzymes involved in this metabolism as adapted from Kaminsky and Zhang (1997) and Zhang *et al.* (1997).



**CYP2C10 is to a lesser extent involved in the 7-hydroxylation of S-warfarin.*

Figure 2.1: Summary of all the metabolites formed from the metabolism of S-warfarin and the enzymes involved in this

As mentioned before, the different CYP450 enzymes metabolise warfarin on different sites of the warfarin molecule, and this is described as its regioselective metabolism (Kaminsky & Zhang, 1997:67). Figure 2.2 is an illustration of the different sites on the *S*-warfarin molecule on which the different CYP450 enzymes act to form the different metabolites as adapted from Kaminsky and Zhang (1997).

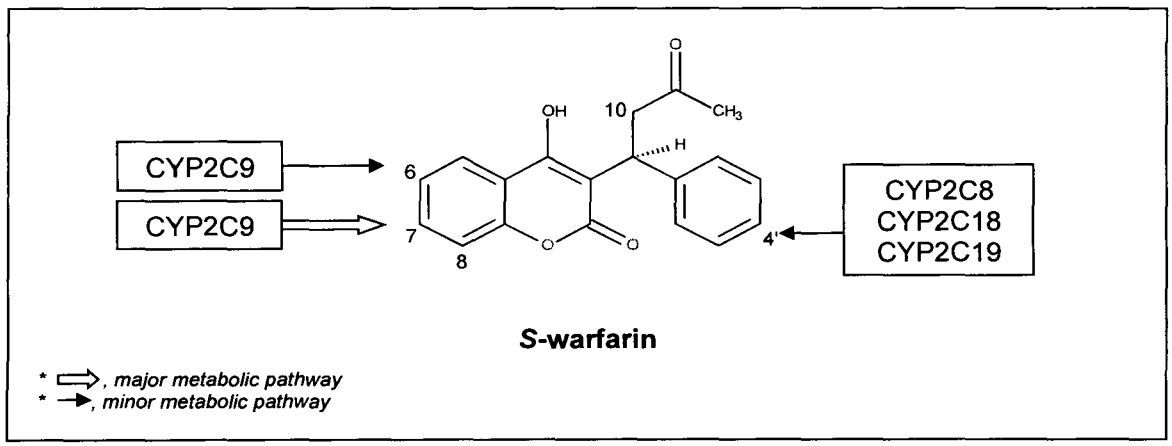
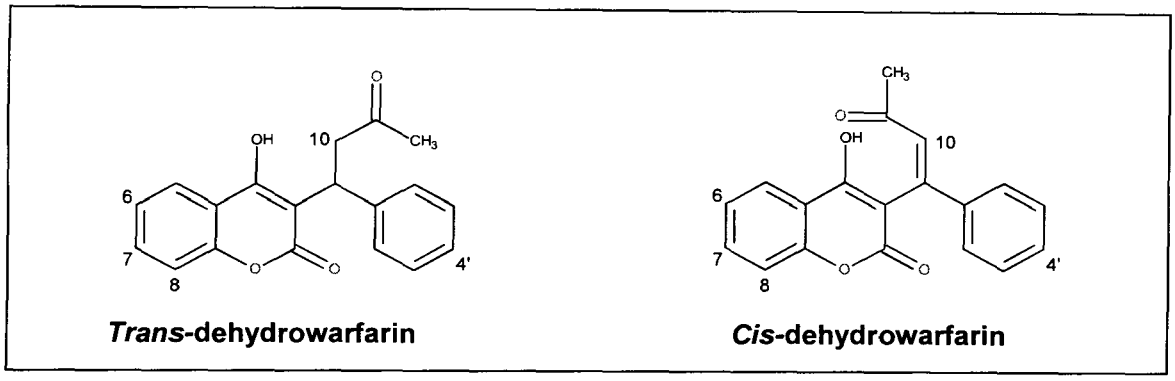


Figure 2.2: The major sites of hydroxylation of S-warfarin catalyzed by CYP450 to produce hydroxylated metabolites

As illustrated by Figure 2.1, CYP3A4 metabolises *S*-warfarin to produce dehydrowarfarin. Dehydrowarfarin has two enantiomers, namely *trans*-dehydrowarfarin and *cis*-

dehydrowarfarin (Kaminsky & Zhang, 1997:68). Figure 2.3 includes illustrations of these two enantiomers as adapted from Kaminsky and Zhang (1997).



***Trans*-dehydrowarfarin** ***Cis*-dehydrowarfarin**

Figure 2.3: Illustration of trans- and cis-dehydrowarfarin, which is formed from the metabolism of S-warfarin by CYP3A4

It is clearly illustrated above that *S*-warfarin is metabolised by a number of CYP450 enzymes. Many other drugs are catalysed by the same CYP450 system, and this may pose a problem. The potential can emerge for many drug interactions between warfarin and other drugs (Kaminsky & Zhang, 1997:68; Zhang *et al.*, 1997:389). Table 2.2 is a list of some of the drugs metabolised by the P450 system as adapted from Kaminsky *et al.* (1997); Guo *et al.* (2006) and Yamazaki & Shimada (1997). These drugs are metabolised by the same CYP450 enzyme subtypes as *S*-warfarin, and this may influence the metabolism of *S*-warfarin. Many warfarin-drug interactions can be caused with these drugs (Kaminsky & Zhang, 1997:71).

Table 2.2: Drugs metabolised by the P450 system that influence the metabolism of S-warfarin and the P450 enzyme subtypes involved in their metabolism

Drug	P450 enzyme subtype
* Diltiazem * Omeprazole Rifampicin	CYP2C19
Diazepam Diclofenac Fluvoxamine Rifampicin Sulfaphenazole Sulfinpyrazone	CYP2C9
* Diltiazem Gestodene Ketoconazole Rifampicin Troleandomycin	CYP3A4

Table 2.3 (continued): Drugs metabolised by the P450 system that influence the metabolism of S-warfarin and the P450 enzyme subtypes involved in their metabolism

Drug	P450 enzyme subtype
Amiodarone Benzbromarone Metronidazole Phenytoin Piroxicam Propafenone Tenoxicam Tetrahydrocannabinol Tienilic acid Tolbutamide Torsemide	Other

** Potential interaction*

As mentioned before, warfarin is administered as a racemate of two enantiomers *R*- and *S*-warfarin (AHFS, 2011; Breccia *et al.,* 2010:e224). A study showed that when warfarin is administered as a racemate, the 7-hydroxylation action of the CYP450 enzyme system was lower than when *S*-warfarin is administered alone without *R*-warfarin, therefore *R*-warfarin might be a competitive inhibitor of *S*-warfarin (Yamazaki & Shimada, 1997:1201; Zhang *et al.,* 1997:390). Another factor that might inhibit the 7-hydroxylation of *S*-warfarin is antibodies. It was found that antibodies against CYP2C9, so-called anti-CYP2C9 immunoglobulin G (IgG), completely repressed the 7-hydroxylation of *S*-warfarin by human CYP450 (Yamazaki & Shimada, 1997:1197).

In conclusion, it is clear that there are multiple factors that influence the metabolism of *S*-warfarin. It was shown that CYP2C9 is the predominant enzyme responsible for the metabolism of *S*-warfarin and that there are numerous warfarin-drug interactions because of this.

- ***The metabolism of R-warfarin***

As mentioned before, the potency of *R*-warfarin is less than that of *S*-warfarin (Anon., 2011; Breccia *et al.,* 2010:e224; Moridani *et al.,* 2006:606). The metabolism of *R*-warfarin is more intricate than that of *S*-warfarin (Guo *et al.,* 2006). There are numerous CYP450 enzymes that are involved in the metabolism of *R*-warfarin, but the two enzymes that are primarily responsible for its metabolism is CYP1A2 and CYP3A4 (Anon., 2011; Kaminsky & Zhang, 1997:69; Guo *et al.,* 2006). The major metabolite produced by CYP1A2 metabolism is 6-hydroxywarfarin, and tCYP3A4 metabolism mainly produces 10-hydroxywarfarin (Anon., 2011; Kaminsky & Zhang, 1997:68, 69). These metabolites are diastereoisomeric alcohols and they are excreted in the urine. These metabolites have very weak anticoagulant activity

(AHFS, 2011). There are several other types of P450 enzymes that participate in the metabolism of *R*-warfarin and these enzymes include CYP1A1, CYP2C18, CYP2C8, CYP2C19 and CYP2C9 (Anon., 2011; Kaminsky & Zhang, 1997:69; Mahajan *et al.*, 2011:11). Some of the primary metabolites formed by CYP3A4 and CYP1A2 are 10-hydroxywarfarin and 6-hydroxywarfarin respectively. There are numerous other metabolites formed by the metabolism of the other CYP enzymes such as 4'-, 7- and 8-hydroxywarfarin (Kaminsky & Zhang, 1997:69; Guo *et al.*, 2006; Miller *et al.*, 2008:2211). Figure 2.4 is a summary of all the metabolites formed from the metabolism of *R*-warfarin and the various enzymes involved in this metabolism as adapted from Kaminsky and Zhang (1997), Yamazaki & Shimada (1997) and Zhang *et al.* (1997).

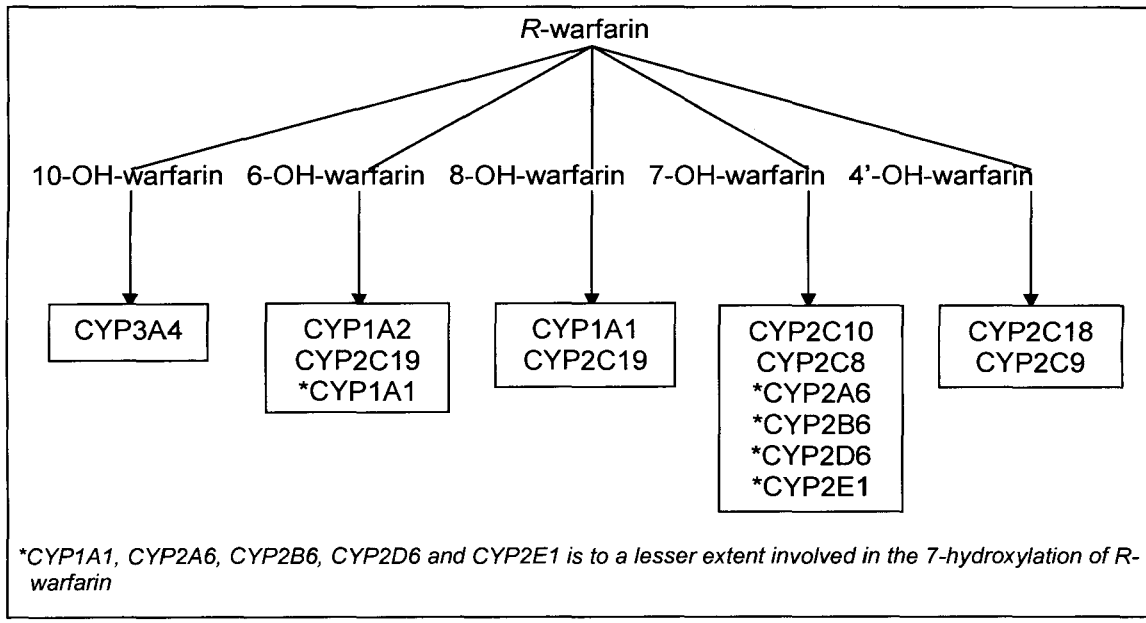


Figure 2.4: Summary of all the metabolites formed from the metabolism of R-warfarin and the enzymes involved in this metabolism

Figure 2.5 is an illustration of the different sites on the *R*-warfarin molecule on which the different P450 enzymes act to form the different metabolites as adapted from Kaminsky and Zhang (1997) and Yamazaki and Shimada (1997).

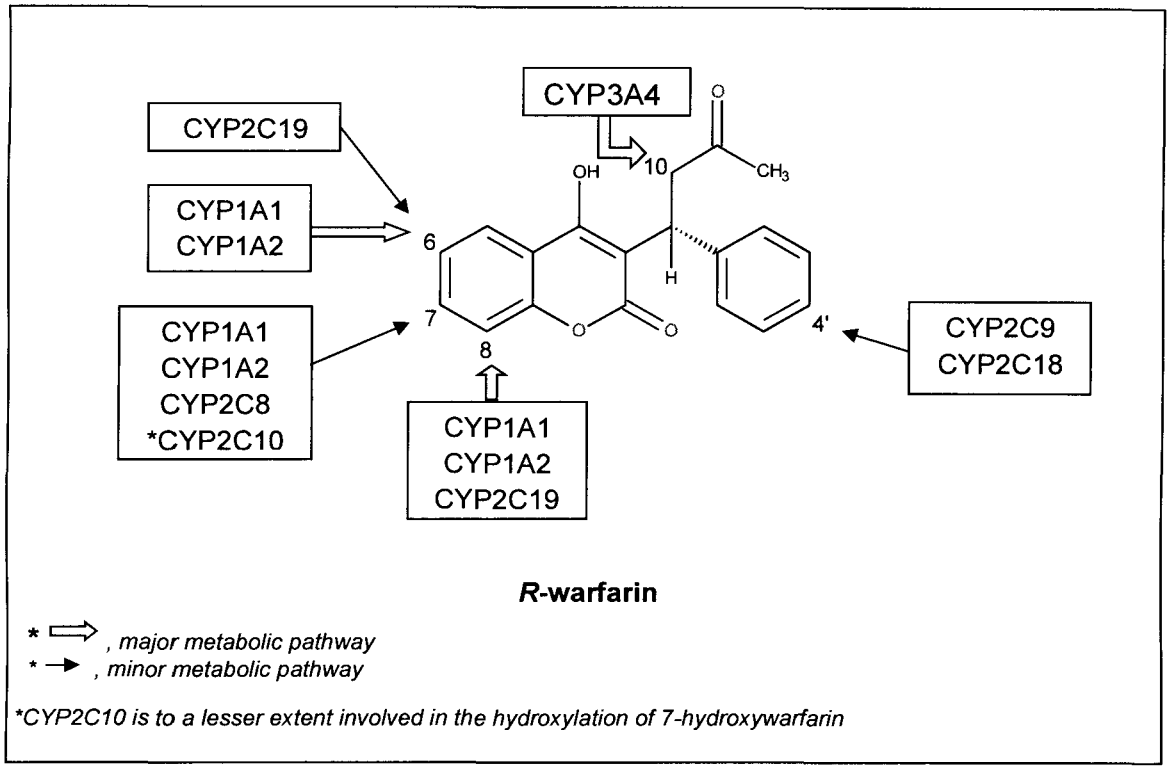


Figure 2.5: The major sites of hydroxylation of R-warfarin catalysed by CYP450 to produce hydroxylated metabolites

There are some of the CYP450 enzyme subtypes that catalyze both *S*-warfarin and *R*-warfarin, but the metabolites that result from this are different. Table 2.3 is a summary of these enzymes and the different metabolites that result from them in the metabolism of *S*-warfarin and *R*-warfarin, as adapted from Kaminsky and Zhang (1997) and Yamazaki and Shimada (1997).

Table 2.4: The P450 enzyme subtypes that catalyse both S-warfarin and R-warfarin and the different metabolites that result from them

Enzyme	*S*-warfarin Metabolites	*R*-warfarin Metabolites
CYP2C9	7-OH-warfarin 6-OH-warfarin	4'-OH-warfarin
CYP3A4	dehydrowarfarin	10-OH-warfarin
CYP2C18	4'-OH-warfarin	4'-OH-warfarin
CYP2C8	4'-OH-warfarin	7-OH-warfarin
CYP2C19	4'-OH-warfarin	6-OH-warfarin 8-OH-warfarin
CYP2C10	7-OH-warfarin	7-OH-warfarin

As seen in the table above (Table 2.3), it is only CYP2C18 and CYP2C10 that produce the same metabolites in both *S*-warfarin and *R*-warfarin. These metabolites are 4'-OH-warfarin and 7-OH-warfarin respectively.

R-warfarin, similar to *S*-warfarin, also has a variety of warfarin-drug interactions (Zhang *et al.,* 1997:390). There are a variety of P450 enzyme subtypes that are the cause of these interactions. The two primary enzyme subtypes responsible for the metabolism of *R*-warfarin is CYP1A2 and CYP3A4 and therefore they play a key role in the warfarin-drug interactions of *R*-warfarin.. Table 2.4 is a summary of all the drugs with which *R*-warfarin has an interaction and the CYP450 enzyme subtypes that play a role in these interactions as adapted from Kaminsky and Zhang (1997), Klein (2001) and Yamazaki and Shimada (1997).

Table 2.5: Drugs metabolised by the P450 system that influence the metabolism of R-warfarin and the P450 enzyme subtypes involved in their metabolism

Drug	P450 enzyme subtype
Dapsone Cyclosporin Cortisol Nifedipine Diazepam Lovastatin Digitoxin Diltiazem Tamoxifen Rifampicin Erythromycin * Gestodene Imipramine Lidocaine Taxol Theophylline Troleandomycin Zonisamide	CYP3A4
Aromatic amines Acetanilide Acetaminophen Aprofloxacin Caffeine Enoxacin Estradiol Ethoxyresorufin Flovoxamine Furaflylline Imipramine	CYP1A2

**Potential interaction*

Table 2.4 (continued): Drugs metabolised by the P450 system that influence the metabolism of R-warfarin and the P450 enzyme subtypes involved in their metabolism

Drug	P450 enzyme subtype
Methoxyresorufin Phenacetin Theophylline α-Naphthoflavone	CYP1A2
Rifampicin Sulfinpyrazone	CYP2C9
#DDC	CYP2A6
Benzbromarone Omeprazole Diazepam Rifampicin	CYP2C19
Cimetidine Propafenone Amiodarone	Other

#*diethyldithiocarbamate*

As with *S*-warfarin, *R*-warfarin also has antibodies that inhibit its 7-hydroxylation. The anti-CYP1A2 IgG antibodies inhibits about 70% of the *R*-warfarin's 7-hydroxylation by the P450 enzymes in the liver (Yamazaki & Shimada, 1997:1197).

In conclusion, there is a variety of CYP450 enzyme subtypes that are involved in the metabolism of *R*-warfarin, but the predominant enzymes are CYP3A4 and CYP1A2. These enzymes also catalyse the metabolism of a number of other drugs, and therefore lead to multiple warfarin-drug interactions.

- ***Other pathways of metabolism of warfarin***

As is illustrated in paragraph 2.2.4.4, warfarin is primarily metabolised by the CYP450 enzyme system in the liver. However, this is only one part of the whole metabolic process of warfarin. There are other enzyme systems that are also involved in its metabolism. It is important to discuss these metabolic pathways as they create a potential for warfarin-drug interactions as well.

As shown in paragraph 2.2.4.4, warfarin is metabolised to hydroxywarfarin by various CYP450 enzymes (Kaminsky & Zhang, 1997:67). These enzymes attach hydroxyl groups on one of five sites on the warfarin molecule. The metabolites mentioned in the above sections (paragraph 2.2.4.4), namely 4'-, 6-, 7-, 8-, and 10-hydroxywarfarins are produced this way (Kaminsky & Zhang, 1997:67; Miller *et al.*, 2009:1239). There is, however, another site on the warfarin molecule where other enzymes act to further metabolise it.

There are certain extrahepatic enzymes that are also involved in the metabolism of warfarin. These enzymes are located in the endoplasmic reticulum and cytosol in the human body. These enzymes are nicotinamide adenine dinucleotide phosphate (NADPH)-dependent carbonyl reductase and they reduce the acetonyl side chain on the warfarin molecule. Diastereoisomeric alcohols are produced by these reactions, which are then excreted in the urine. These excretions form an important part of the clearance of warfarin, as 15-20% of warfarin that is administered orally, is cleared in this way. Not many studies have been done on these enzymes and as a result these enzymes have not all been characterised. However, it is known that these enzymes appear to be stereoselective (Kaminsky & Zhang, 1997:72; Coffman *et al.,* 1998:73; Green *et al.,* 1998:507). Figure 2.6 illustrates the acetonyl side chain on the warfarin molecule, which is reduced by the NADPH-dependent carbonyl reductase as adapted from Miller *et al.* (2009).

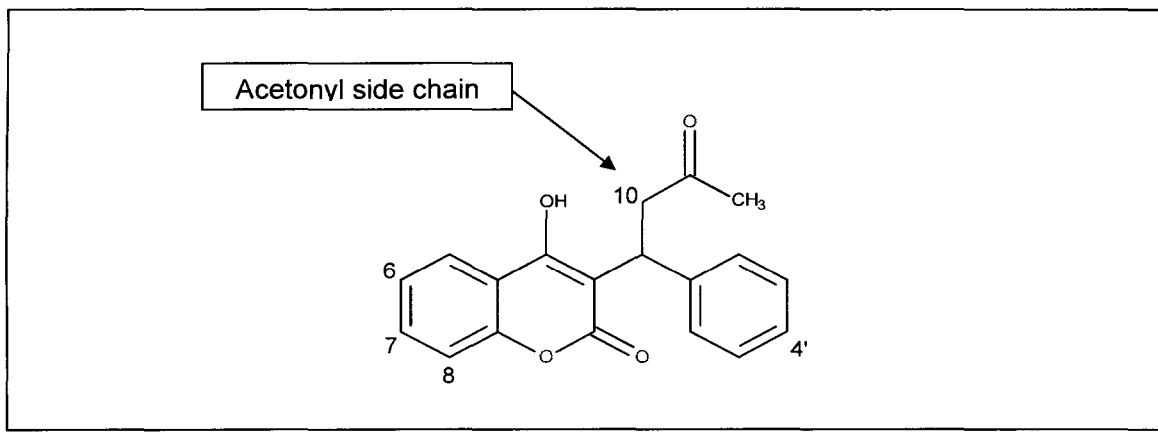


Figure 2.6: A schematic representation of warfarin and the acetonyl side chain which is reduced by the NADPH-dependent carbonyl reductase

There is another way in which warfarin is metabolised. The products from its phase I metabolism are substrates for phase II metabolism (Miller *et al.,* 2009:1239). Phase I metabolism is necessary for the hydroxylation of the warfarin molecule to produce hydroxywarfarins. These hydroxywarfarins are then inactive metabolites and therefore do not show any pharmacological activity (Miller *et al.,* 2008:2211). However, they are apt substrates for phase II metabolism. The reactions of phase II metabolism are catalyzed by a vast family of uridine diphosphate (UDP) glucuronosyltransferase (UGTs) enzymes. These enzymes bind UDP glucuronic acid molecules to other molecules to make them more water soluble. These conjugated molecules are therefore more polar and are excreted in the urine or bile more effectively (Coffman *et al.,* 1998:73; Miller *et al.,* 2008:2211-2212; Xu *et al.,* 2005:250). By removing these hydroxywarfarins, any remaining pharmacological activity will

be removed as well. In addition, any inhibition caused by the hydroxylated warfarins will be eliminated (Miller *et al.,* 2008:2212).

It is obvious then that glucuronidation is a very important step in the elimination of warfarin.

There are a number of so-called "superfamilies" of conjugating enzymes involved in the phase II metabolism of various compounds. These include the sulfotransferases (SULTs) and UDP-glucuronosyltransferases (UGTs), the DT-diaphorase or otherwise known as the NAD(P)H:quinine oxidoreductase (NQOs) or NAD(P)H:menadione reductase (NMOs). Other families of conjugating enzymes are the epoxide hydrolases (EPHs), glutathione *S*-transferases (GSTs) and lastly the *N*-acetyltransferases (NATs) (Xu *et al.,* 2005:250). These "superfamilies" are responsible for the phase II metabolism of a wide range of compounds that bear a range of functional groups. These functional groups include hydroxyl, carboxylic, amine or thiol groups (King *et al.,* 1996:92; Zielinska *et al.,* 2008:140).

The two principle "superfamilies" that will be highlighted are the sulfontransferases and the UDP-glucuronosyltransferases. These two families of conjugating enzymes are important as they catalyze sulfonation and glucuronidation in molecules that contain the hydroxyl functional group (Xu *et al.,* 2005:250). However, not much is known about sulfonation (Jones *et al.*, 2010). As mentioned in paragraph 2.2.4.4, raceamic warfarin is hydroxylated to 4'-, 6-, 7-, 8- and 10-hydroxywarfarins, and therefore are suitable substrates for glucuronidation (Coffman *et al.,* 1998:73; Kaminsky & Zhang, 1997:67; Miller *et al.,* 2008:2211-2212; Miller *et al.* 2009:1239;; Xu *et al.*, 2005:250).

There is still much research that needs to be conducted on phase II metabolism of warfarin. The only models available are rat models. The only "human" models available are that of *in vivo* studies (Kaminsky & Zhang 1997:72). There are slight differences in the conjugated metabolites formed between rats and humans, and this poses a problem. As early as 1992 Jansing and colleagues discovered that different hydroxywarfarins were conjugated differently by rat hepatocytes. They found that 4'-hydroxywarfarin was the primary substrate for glucuronidation and that 6-hydroxywarfarin was primarily sulphated. They also showed that 7- and 8-hydroxywarfarin were both glucuronated and sulphated (Jansing *et al.,* 1992). Later, Kaminsky and Zhang (1997:72) confirmed this by obtaining the same results from their study. Further, they went on and mentioned that in a previous *in vivo* study it was found that 10-, 8- and 6-hydroxywarfarin was conjugated in humans. In a later study done by Zielinska *et al.* (2008:143), it was found that 4'- and 10-hydroxywarfarin was almost completely unconjugated in humans. It was confirmed more recently that the conjugates formed by rat hepatocytes are 4'-, 7-, and 8-hydroxyglucuronides. The conjugates formed by the human liver are 6-, 7-, and 8-hydroxyglucuronides (Jones *et al.,* 2010). It is also

important to note that warfarin itself is not a substrate for glucuronidation (Zielinska *et al.*, 2008:145).

As with the CYP450 system, the glucuronidation enzymes are also composed of a variety of isoforms. All the UDP-glucuronosyltransferase (UGT) enzymes are divided into two major subfamilies, namely UGT1 and UGT2 (King *et al.,* 1996:92). There are over 12 to 40 different isoforms of UGTs in these subfamilies (Coffman *et al.,* 1998:73; Green *et al.*, 1998:507; King *et al.,* 1996:92). All of these isoforms are substrate specific. These isoforms are located hepatically and extrahepatically (Zielinska et al., 2008:140). Of all the isoforms, UGT1A10 is the most important isoform involved in the glucuronidation of hydroxywarfarins. The UGT1A10 isoform exhibits the highest activity towards the most hydroxywarfarins (Jones *et al.,* 2010; Miller *et al.,* 2008:2215; Zielinska *et al.,* 2008:146). There are other UGT isoforms involved in the metabolism of warfarin that are more stereospecific. These include UGT1A1, UGT1A3, UGT1A8 and UGT1A9 (Jones *et al.,* 2010; Zielinska *et al.,* 2008:143). The three major hydroxywarfarins, 6-, 7-, and 8-hydroxywarfarin, are glucuronidated to various extents by these UGT isoforms. According to Zielinska *et al.*, (2008:141, 143), UGT1A10 shows very weak activity towards 4'-hydroxywarfarin in humans. Table 2.5 is a summary of these hydroxywarfarins and the UGT isoforms involved in their glucuronidation as adapted from Jones *et al.* (2010), Miller *et al.* (2008) and Zielinska *et al.* (2008).

Table 2.6: Summary of the hydroxywarfarins that are substrates for the glucuronidation in humans and the UGT-isoforms involved in their glucuronidation

Hydroxywarfarin	UGT-isoform
6-hydroxywarfarin	UGT1A1, UGT1A3, UGT1A10, *UGT1A8
7-hydroxywarfarin	UGT1A1, UGT1A3, UGT1A10, *UGT1A8
8-hydroxywarfarin	UGT1A1, UGT1A3, UGT1A8, UGT1A9, nUGT1A10

**: UGT1A8 is to a much lesser extent involved in the glucuronidation of 6-, and 7-hydroxywarfarin*

n: UGT1A10 is to a large extent responsible for the glucuronidation of 8-hydroxywarfarin

Figure 2.7 indicates the sites on the hydroxywarfarin molecules where glucuronidation takes place as adapted from Zielinska *et al.* (2008).

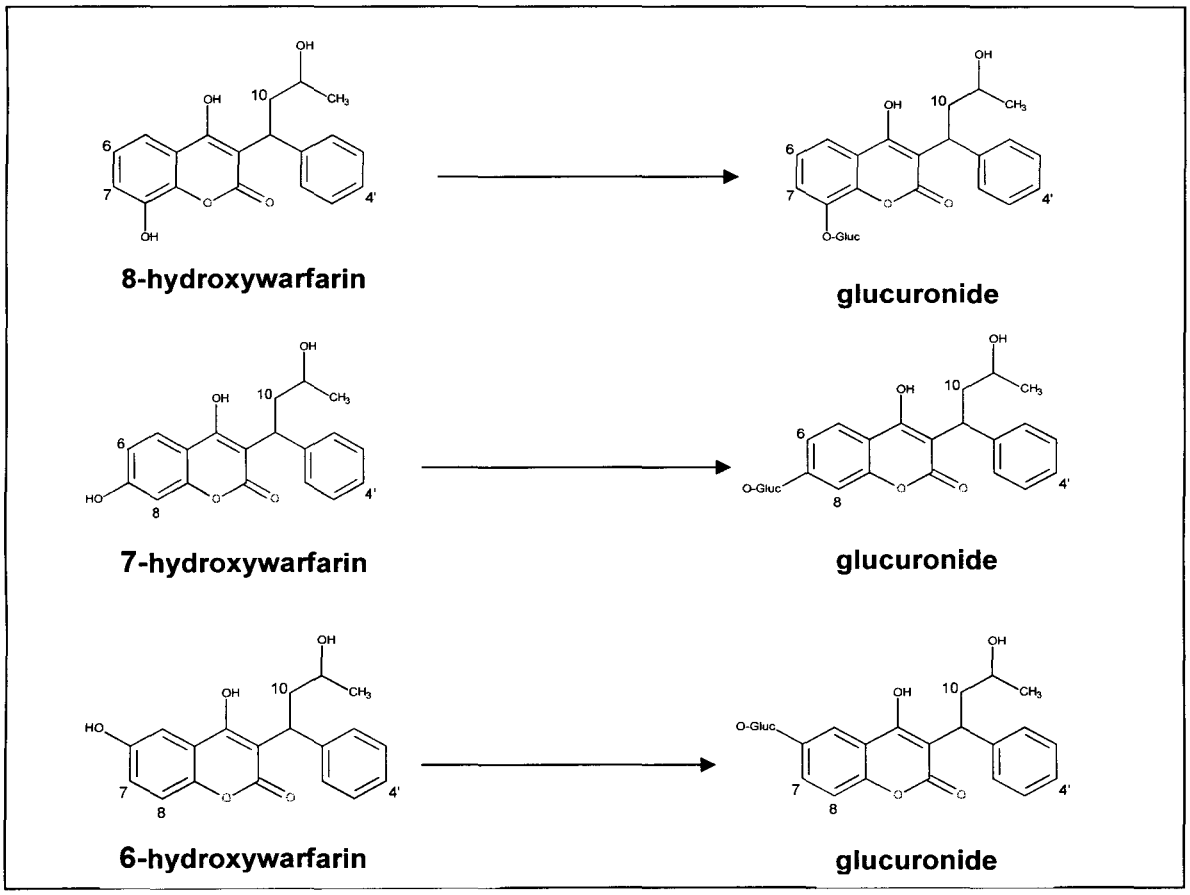


Figure 2.7: Illustration of the sites on the hydroxywarfarin molecule where glucuronidation takes place

It is interesting to note that both UGT1A10 and UGT1A8 are found in the intestine. Another enzyme isoform found in the intestine is the CYP2C19 isoform of the CYP450 enzymes. CYP2C19 hydroxylates *R*-warfarin to 8-hydroxywarfarin, which is the primary substrate for UGT1A10 and UGT1A8. Therefore, these two mechanisms of metabolism work together in the intestine. The reason why *R*-warfarin is not as potent as *S*-warfarin may be explained by this (Zielinska *et al.,* 2008:146, 147).

The reason why phase II metabolism of warfarin is so important is because the isoforms involved in this metabolism is also involved in the phase II metabolism of other compounds (Green *et al.,* 1998:507). Table 2.6 is a summary of the UGT-isoforms involved in the phase II metabolism of warfarin as well as in the phase II metabolism of other compounds as adapted from Green *et al.* (1998) and Miller *et al.* (2008).

Table 2.7: The UGT-isoforms involved in the phase II metabolism of warfarin and the other compounds which they metabolise

UGT-isoform	Compound
1A1	Anthraquinones Bilirubin Certain oestrogens Flavonoids Oripavine opioids Other coumarins Phenolic compounds
1A3	2-acetylaminofluorene metabolites 2-hydroxyestrone Estrone Hydroxy benzo[*a*]pyrene metabolites
1A9	Aliphatic alcohols Anthraquinones Bulky phenols Flavonoids *NSAIDs
1A10	Phenolic oestrogens

**Non-steroidal anti-inflammatory drugs*

2.2.4.5 Warfarin resistance

It was mentioned earlier that some forms of anticoagulants are used as rodenticides. These anticoagulants fall in the same pharmacological group as warfarin (Brunton, 2012). Early studies already found that warfarin resistance is developing in rats (MacNicoll, 1985). MacNicoll already discovered in 1985 that some rats are susceptible to the effects of warfarin and some rats are resistant. He speculated that the resistance was due to a decreased affinity of the enzymes in the liver for warfarin and vitamin K_1 2,3-epoxide. These rats required higher warfarin doses to achieve an anticoagulant effect than those rats that are not resistant (MacNicoll, 1985). In later studies it was discovered that there might be different types of resistance that can develop towards warfarin in rats. Misenheimer and Suttie (1990) found that resistance can be due to reversible inhibition of warfarin in resistant rats, vs. irreversible inhibition of the epoxide reductase in normal rats. They also showed that there could be differences in warfarin clearance in different rats. In 2001, Linder classified two possible mechanisms of warfarin resistance. He proposed that there is a pharmacokinetic and pharmacodynamic mechanism of warfarin resistance. The pharmacokinetic mechanism is due to a more rapid clearance rate of *S*-warfarin compared to

control subjects. The cause of this is unclear, but it can be due to a mutant type duplication or "muti-duplication" of CYP450 enzymes that leads to an "ultra-rapid metabolism phenotype". The pharmacodynamic mechanism is possibly due to an increased response to the anticoagulant reversal by vitamin K. Linder tested warfarin-resistant individuals stabilised on 75 mg warfarin per day and found that when administered with 0.5 mg of vitamin K, the activity of prothrombin raised from 25% to 43%. He speculated that this was due to an increase in the affinity of the vitamin K_1 receptor (Linder, 2001:13).

Another form of resistance to warfarin can be due to different sensitivities towards its anticoagulant effect. The genetics of CYP2C9 is responsible for the metabolism of mainly *S*-warfarin to its inactive metabolites. Several polymorphisms of this enzyme exists that influences warfarin dose requirements (Wells *et al.,* 2010:e259). The genotyping of CYP2C9 produced six alleles (CYP2C9*1, CYP2C9*2, CYP2C9*3, CYP2C9*4, CYP2C9*5 and CYP2C9*6) present in humans, where the "wild-type" allele is CYP2C9*1. Compared to these "wild-type" individuals identified with alleles *2 and *3 normally presented with requiring a lower warfarin dose (Linder, 2001:11; Scott *et al.,* 2008:495; Wells *et al.,* 2010:e259). Some individuals who presented with homozygous alleles of CYP2C9*1 required higher warfarin doses than normal (Linder, 2001:11).

Another enzyme that presents with polymorphisms is vitamin K epoxide reductase (VKOR). The VKOR enzyme is responsible for the conversion of vitamin K hydroquinone into vitamin K 2,3-epoxide in the vitamin K cycle. An essential protein responsible for the enzymatic activity of VKOR was encoded in 2004 and was named the vitamin K epoxide reductase subunit 1 (VKORC1) (Wang, *et al.,* 2008:90). It is known that VKOR works in two places in the vitamin K cycle. It converts vitamin K_1 to the reduced form of vitamin K (vitamin K_1H_2). Vitamin K_1H_2 is then the co-factor needed for the synthesis of active coagulation factors. Vitamin K 2,3-epoxide is converted to vitamin K_1, also by VKOR. Only this conversion by VKOR is sensitive to the inhibitory effects of warfarin (Linder, 2001:10; Wang *et al.,* 2008:90). Figure 2.8 is a representation of the vitamin K cycle and the locations of action of VKOR in the cycle as adapted from Linder (2001).

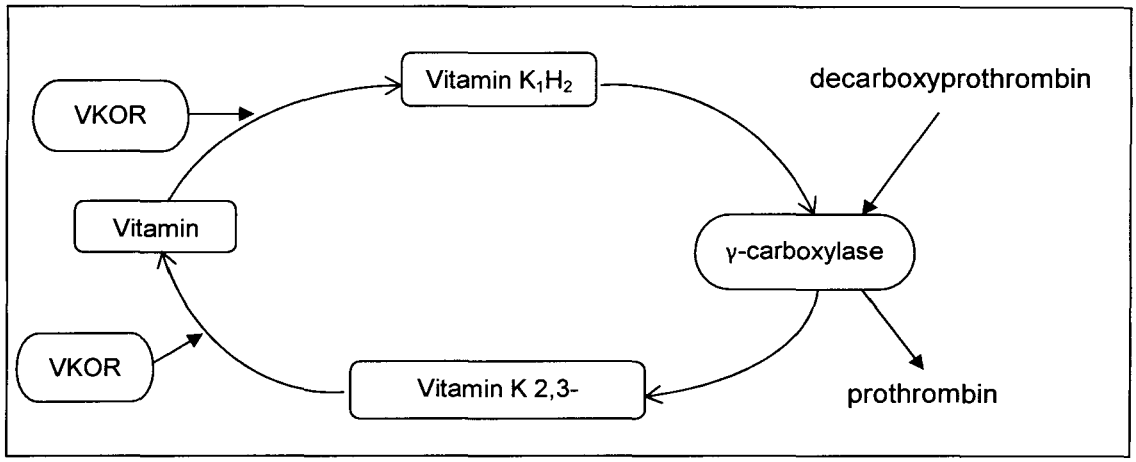


Figure 2.8: The vitamin K cycle and the sites of action of VKOR

VKORC1 activity can be greatly affected by gene mutations of its amino acids. Wang and colleagues found that the substitution of just a single amino acid in VKORC1 can result in VKORC1 being completely inactive, thus diminishing the effects of warfarin (Wang *et al.,* 2008:94). Different polymorphisms of VKORC1 also influence warfarin dosing. Some polymorphisms are linked to decreased warfarin dosing requirements. Some of these polymorphisms include the 1173 C>T intronic polymorphism and the -1639 G>A exonic polymorphism (Wells *et al.,* 2010:e259). Scottt and colleagues also identified different polymorphisms in VKORC1. With these polymorphisms they identified different alleles responsible for the varied warfarin response to VKORC1. An example of one of these alleles is the g.-1639G →A promoter allele that causes a reduction in the expression of VKORC1, which leads to a decreased warfarin dose requirement. Other alleles responsible in VKORC1 that change warfarin requirements is the g.-1639G promoter allele, which is linked to normal warfarin doses and the g.-1639A promoter allele, which is linked to lower warfarin doses (Scott *et al.,* 2008:496-497).

There are other factors that also influence warfarin dosing besides genetic factors. These factors include "increasing age, low body weight, Caucasian race, liver disease, tobacco use, lack of physical exercise, low dietary vitamin K intake and certain concomitant medications". These factors are associated with a lower warfarin dose requirement (Wells *et al.,* 2010:e260).

The genotyping of CYP2C9 and VKORC1 is important as this is used to individualise warfarin dosing in patients. The research done on pharmacogenetic warfarin dosing is used to develop algorithms to predict the warfarin dose of an individual. These algorithms

incorporate clinical and genetic factors to predict these individual doses by means of regression equations (Finkelman *et al.,* 2011:612). Wells and colleagues (2010) give an example of such an algorithm that can be used to obtain a patient's warfarin dose using the clinical and genomic characteristics of a patient. The algorithm constitutes the following:

*Dose=1.85-0.048(age)+0.041(BMI)+0.05(height in cm)-0.73(less exercise)-1.13(2C9*2 hetero)-2.09(2C9*2 homo)-1.51(2C9*3 hetero)-1.43(VKORC1 GA)-2.86(VKORC1 AA)-1.33(4F2 CC)-1.24(4F2 CT)-1.46(angiotensin II receptor antagonist)-0.84(β-blockers)* (Wells *et al.,* 2010:e262*).*

As can be seen above, these algorithms are complicated and sometimes need a computer or a web-based application to calculate the doses. However they are very accurate. To simplify this, the U.S. Food and Drug Administration (FDA, 2010) included a table in the warfarin product label that can be used to determine the estimated warfarin dose according to the genotype of the patient. These tables show to be very accurate in predicting the warfarin dose as well (Cho *et al.,* 2011:1372; Finkelman *et al.,* 2011:612, 615). Table 2.7 is an example of the table published by the FDA in the warfarin product label as adapted from FDA (2010) and Finkelman, *et al.* (2011).

Table 2.8: Example of the table in the warfarin label to predict the individual dose of warfarin based on clinical an genetic factors

FDA Label: Range of expected therapeutic warfarin doses (mg/day) based on CYP2C9 and VKORC1 genotypes						
VKORC1-1639	**CYP2C9*1*1**	**CYP2C9*1*2**	**CYP2C9*1*3**	**CYP2C9*2*2**	**CYP2C9*2*3**	**CYP2C9*3*3**
GG	5-7	5-7	3-4	3-4	3-4	0.5-2
GA	5-7	3-4	3-4	3-4	0.5-2	0.5-2
AA	3-4	3-4	0.5-2	0.5-2	0.5-2	0.5-2

2.2.4.6 Elimination

A great part of the elimination of warfarin is its metabolism. However, there are other factors besides enzymes that play a role in the elimination of warfarin.

The elimination half-life of warfarin differs between individuals. The average half-life is about 40 hours whereas the range can be anything between 20 and 60 hours (AHFS, 2011).

The plasma half-life of warfarin is not dependent on the dose administered (AHFS, 2011). It ranges from 15 to 85 hours as this also differs between individuals. The average plasma

half-life is about 42 hours. However, these values decrease in patients with renal disease (Moffat *et al.,* 2011). Other sources state that the plasma half-life can also be about 37 hours (Sweetman, 2011).

The half-lives of the two enantiomers of warfarin, *R*- and *S*-warfarin, also differ. The half-life of *R*-warfarin is much longer than that of *S*-warfarin. The half-life of *R*-warfarin ranges from 37 to 89 hours, where the half-life of *S*-warfarin is anything between 21 and 43 hours (AHFS, 2011; Moffat *et al.,* 2011). The average half-lives are 45 hours and 30 hours respectively for *R*-warfarin and *S*-warfarin (Moffat *et al.,* 2011). The clearance between the two enantiomers also differs. The clearance of *R*-warfarin is 50% less than that of *S*-warfarin (AHFS, 2011). It is estimated that the clearance of raceamic warfarin is anything between 1.16 to 4.35 ml/hr/kg of body weight. The free fraction of warfarin in the serum correlates greatly with this clearance tempo (Yacobi *et al.,* 1976).

The renal clearance also differs between warfarin metabolites. *S*-7-hydroxywarfarin clears at a rate of 182 to 583 ml/min in the kidneys. The renal clearance for the *R,S*-warfarin alcohol is considerably less and achieves a clearance rate of only 23 to 191 ml/min. The rates of the renal clearance of these metabolites are far more extensive than that of its glomerular filtration. The renal clearance was far less when phenylbutazone was administered with warfarin (39 to 62 ml/min for *S*-7-hydroxywarfarin and 5 to 17 ml/min for *R,S*-warfarin alcohol). Phenylbutazone inhibits the renal secretion of these metabolites (Chan *et al.,* 1994).

Protein binding also influences the elimination of warfarin. The protein-binding sites in the protein molecule are not specific. Any drug can bind on this protein molecule. There is a great chance that one drug can be displaced by another, especially if it is a highly protein-bound drug. The displacements of one drug by another cause the free-fraction of that drug to increase in the blood plasma. An increased elimination of the free drug results from this, thus for a highly protein-bound drug such as warfarin (which is about 97% to 99% bound to protein), this can play a major role in its elimination (BNF, 2011; Moffat *et al.*, 2011; Yacobi *et al.,* 1976).

Finally, many sources agree that the metabolites of warfarin are excreted mainly in the urine (AHFS, 2011; Moffat *et al.,* 2011; Sweetman, 2011). There is a small fraction (<1%) however, of the unchanged drug that is excreted in the urine (Moffat *et al.*, 2011). Another path of excretion is the bile (Moffat *et al.,* 2011; Sweetman, 2011). Very little of the drug is excreted in the faeces (Powell *et al.,* 1977).

2.2.5 PHARMACODYNAMICS

2.2.5.1 Mechanism of action

Before warfarin's mechanism of action can be understood, attention must first be given to the process of coagulation.

The coagulation process is a cascade of reactions catalyzed by a number of enzymes. Each reaction activates a corresponding protein, which in turn is the co-enzyme for the next reaction (Jay & Lui, 2006:31). The result of this coagulation cascade is the conversion of inactive fibrinogen to active fibrin that will eventually lead to the formation of a blood clot (Jay & Lui, 2006:34). The proteins involved in this cascade are called coagulation factors that circulate freely in the blood plasma (Jay & Lui, 2006:31).

The coagulation cascade is divided into two pathways, the extrinsic pathway and the intrinsic pathway. The reason for this is because the two pathways are initiated separately by different mechanisms (Jay & Lui, 2006:31). Coagulation is started via the extrinsic pathway when Factor VII is converted to its active form VIIa when it binds to exposed tissue factor (TF) on the subendothelial cells after injury of the vascular tissue (Brunton, 2012). The TF-VIIa complex directly activates Factor X by converting it to Factor Xa. Finally, this leads to the conversion of inactive prothrombin to active thrombin (Factor II to Factor IIa), which catalyzes the reaction that converts fibrinogen to fibrin (Jay & Lui, 2006:31, 34).

Factor XII, prekallikrein, and high-molecular-weight kininogen interacts with kaolin and other surfaces to form small amounts of active Factor XIIa. The activation of Factor XI to XIa results from this. Factor XIa then causes the activation of Factor X to Xa (Brunton, 2012). Here the intrinsic pathway "meets" the extrinsic pathway (Jay & Lui, 2006:31). The activation of Factor X by Factor IXa is enhanced by Factor VIIa, phospholipids and calcium (Brunton, 2012). The prothrombinase complex which catalyzes the conversion of prothrombin to thrombin is formed by Factor Xa, Factor Va, calcium and platelet phospholipids (Jay & Lui, 2006:34). The activation of Factor XI is not known at this stage (Brunton, 2012). Finally, due to the action of thrombin, fibrinogen is changed to fibrin (Jay & Lui, 2006:34). Figure 2.9 is a graphical representation of the coagulation cascade as adapted from Jay and Lui (2006).

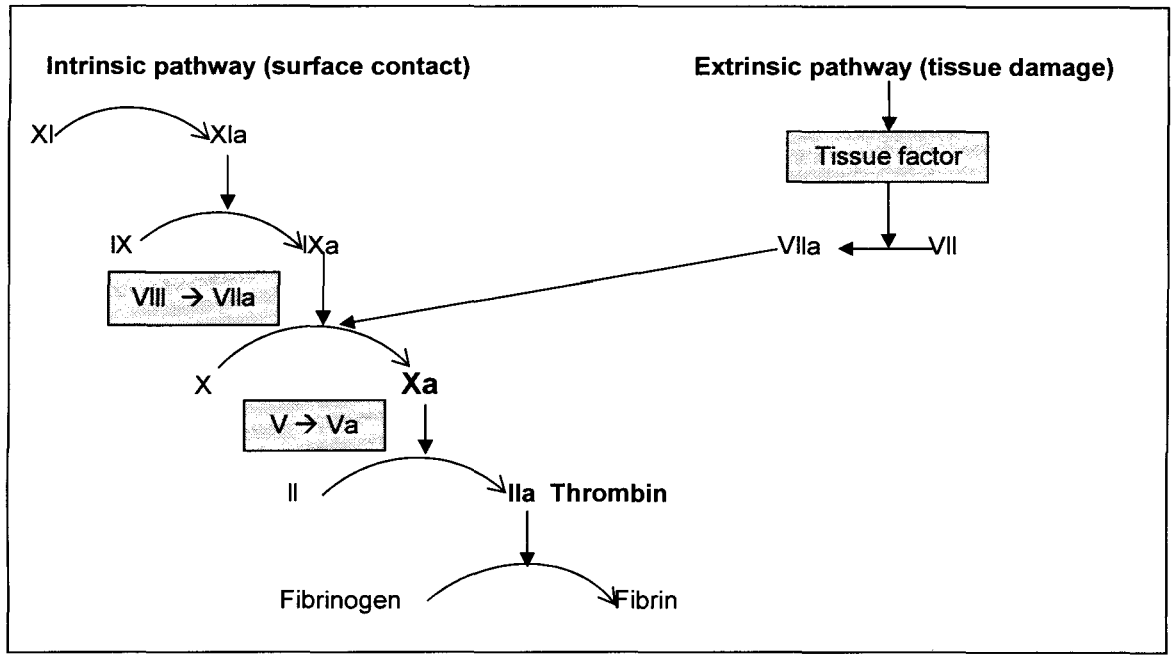


Figure 2.9: The coagulation cascade

Table 2.8 shows all the blood clotting factors and their synonyms as adapted from Katzung *et al.* (2009).

Table 2.9: Blood clotting factors

Factor	Synonym
I	Fibrinogen
II	Prothrombin
III	Tissue thromboplastin
IV	Calcium
V	Proaccelerin
VII	Proconvertin
VIII	Antihemophilic factor (AHF)
IX	Christmas factor, plasma thromboplastin component (PTC)
X	Stuart-Power factor
XI	Plasma thromboplastin antecedent (PTA)
XII	Hageman factor
XIII	Fibrin-stabilizing factor

Some of these coagulation factors, factors II, VII, IX and X, together with the anti-coagulant proteins C, S and Z, are mainly synthesised in the liver (Batty & Smith, 2010:243; Brunton, 2012; Van Goor *et al.*, 2008:214). These coagulation factors and anti-coagulant proteins are

in their inactive form and need to be changed into their biological active forms. The change from inactive form to active form is achieved via γ-carboxylation of glutamyl residues in these cogulation factors by the reduced form of vitamin K (vitamin KH_2) (Hirsh *et al.,* 2003:1633; Watanabe *et al.,* 2010:114). In this reaction vitamin KH_2 is converted to its epoxide form (vitamin KO) by γ-glutamyl carboxylase (Mahajan *et al.,* 2011:11). This reaction is coupled to the carboxylation of glutamate residues (Glu) to γ-carboxyglutamates (Gla) that is, the inactive forms of the coagulation factors and anti-coagulant proteins to their active forms in the endoplasmic reticulum (Brunton, 2012; Hirsh *et al.,* 2003:1633). The coagulation factors and anti-coagulant proteins are all vitamin K-dependent proteins. These vitamin K-dependent proteins also include prothrombin, thus the γ-carboxylation also includes the conversion of inactive prothrombin to active thrombin (Watanabe *et al.,* 2010:115). After this vitamin KO must be quickly changed back to vitamin KH_2 for the cycle to repeat itself (Mahajan *et al.,* 2011:11). Vitamin KO is changed to vitamin K_1 by the enzyme vitamin KO reductase. Vitamin K_1 is also obtained from the diet. Vitamin K_1 is then converted to vitamin KH_2 by the enzyme vitamin K reductase. Vitamin KO reductase is sensitive to warfarin, whereas vitamin K reductase is not. Warfarin therefore inhibits the formation of vitamin K_1 from vitamin KO. Eventually this causes a reduction in the formation of vitamin KH_2 which is responsible for the carboxylation of the glutamate residues in the coagulation factors (Hirsh *et al.,* 2003:1634). The carboxylation of the glutamate residues is then incomplete, resulting in coagulation factors having weaker coagulation activity (Hirsh *et al.,* 2003:1633). A decrease in the blood plasma concentrations of these coagulation factors leads to a retarded blood clotting response and therefore an increased tendency to bleed (Koch & Biber, 2007:41). The effects of warfarin can be overcome by administering a low dose of vitamin K_1. Vitamin K_1 bypasses the vitamin KO reductase enzyme and thus the effects of warfarin. It can then be converted to vitamin KH_2 as the vitamin K reductase enzyme is relatively resistant to warfarin (Hirsh *et al.,* 2003:1633, 1634). Figure 2.10 shows the link between the vitamin K cycle and γ-carboxylation of glutamate residues as adapted from Hirsh *et al.* (2003).

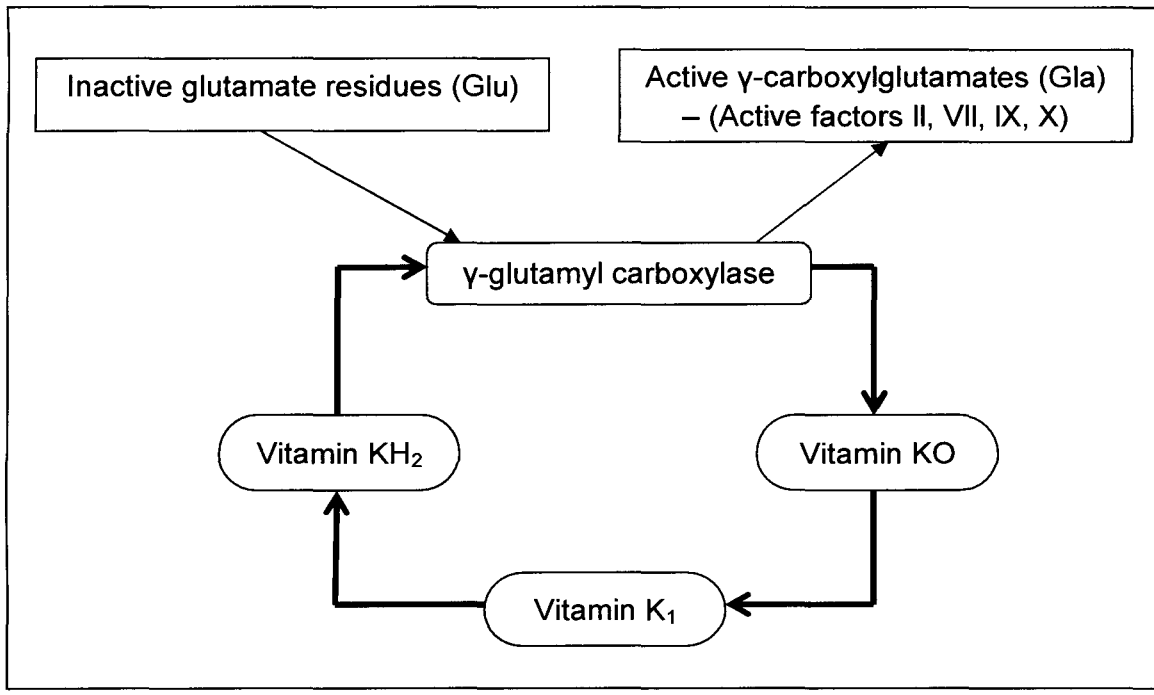


Figure 2.10: The link between the vitamin K cycle and the γ-carboxylation of glutamate residues

2.2.5.2 Side-effects

As warfarin is an anticoagulant, the most common and serious side-effect experienced with warfarin therapy is bleeding (Tanaka *et al.,* 2008:118). It is estimated that minor bleeding will occur in 15% of patients receiving warfarin therapy per year (Leiker *et al.,* 2009:227). Major bleeding episodes will occur in approximately 1.1% to 2% of patients on chronic warfarin therapy (Tseng *et al.,* 2010:939; Wang *et al.,* 2008:90; Wiedermann & Stockner, 2008:S13). These percentages seem small, but it is quite serious. Olsen *et al.* noted in 2010 that during 2008 about 4.1% of the Danish population in an age group of 65 to 69 years of age collected prescriptions for anticoagulants (Olsen *et al.,* 2010:297). Wiedermann and Stockner stated in 2008 that more or less 1% of the European population is on anticoagulant therapy (Wiedermann & Stockner, 2008:122). Therefore, this can be seen as a serious side-effect.

Bleeding usually occurs in patients older than 65 years. These patients usually have a history of gastrointestinal bleeding or stroke (Tanaka *et al.,* 2008:118). There is no official definition for bleeding, but bleeding can be divided into three groups according to severity namely fatal, major or minor bleeding. Wiedermann and Stockner categorised different forms of bleeding into these three groups (Wiedermann & Stockner, 2008:S14). Table 2.9 is an example of this categorisation adapted from Wiedermann and Stockner (2008).

Table 2.10: The three different groups of bleeding and their examples

SEVERITY OF BLEED	DIFFERENT TYPES OF BLEEDING
Fatal	Death caused by hemorrhage
Major	Intracranial bleed Retroperitoneal bleed Intraocular bleed Muscle and compartment syndrome Invasive procedure required to stop bleeding Active bleeding from any orifice and systolic blood pressure lower than 90mmHg, oliguria, or a more than 2g fall in haemo-globin
Minor	Any other form of bleeding

Bleeding is treated differently according to the severity of the bleed. The treatment of bleeding is also determined by the International Normalised Ratio (INR) of the patient. There are mainly three ways of treating a bleed:

- Discontinue the use of the vitamin K antagonist
- Administer oral or intravenous vitamin K (Wiedermann & Stockner, 2008:S15)
- Substitute clotting factors, which include fresh frozen plasma (FFP), prothrombin complex concentrates (PCC) or recombinant activated factor VII (rFVIIa) (Tanaka *et al.,* 2008:118; Wiedermann & Stockner, 2008:S15)

With the treatment of minor bleeding, the use of the anticoagulant only needs to be discontinued. For major bleeding, the functional coagulation factors must be administered as quickly as possible (Wiedermann & Stockner, 2008:S15).

As seen in table 2.9, bleeding (or haemorrhage) can occur in different locations in the body. The severity of the bleed is often also dependent on the location of the bleed. It is well documented in literature that the risk for brain haemorrhage increases when oral anticoagulants are used. Therapy with anticoagulants is also linked to lobar and thalamic haemorrhages (Itabashi *et al.,* 2009:87). Intracranial haemorrhage is a serious bleeding complication as this is the main cause of morbidity and mortality. The risk for intracranial haemorrhage increases with a head injury sustained by patients on anticoagulant therapy (Siracuse *et al.,* 2010:724, 725, 727). To emphasise how serious this complication is, Fang *et al.* (2007) did a cohort study on the number of deaths and disabilities that resulted from

warfarin therapy in patients with atrial fibrillation as a result of intracranial haemorrhage. They found that 76% of patients on warfarin therapy either had a disability or died from an intracranial haemorrhage (Fang *et al.,* 2007). Anticoagulants also increase the risk of extracerebral intracranial haemorrhage. These types of haemorrhages include subdural, epidural and subarachnoid haemorrhages. However, Olsen and his colleagues (2010) found that there is no link between anticoagulant therapy and subarachnoid haemorrhage (Olsen *et al.,* 2010:297, 298).

Haemorrhaging can also occur in the eye. Bleeding in the eye can occur in various ways, including hyphemas, bloody tears or subconjunctival, vitreal, retinal or choroidal haemorrhages. Warfarin treatment sometimes results in angle closure glaucoma as well. Subretinal and retinal haemorrhages are also a common complication of warfarin therapy. Subconjunctivital haemorrhage is often the cause of panic as the appearance of this is worrying. It causes no symptoms or sight disturbances and it will clear up in 5 to ten days (Bodack, 2007:113, 116; Leiker *et al.,* 2009:228).

Another location of haemorrhage in the body is the intestine. However, an uncommon complication of warfarin is that 1 in 2500 of reported cases is of spontaneous intramural intestinal haematoma. The symptoms of this warfarin-related complication are very similar to that of acute abdomen. Surgical intervention is usually the result (Tseng *et al.,* 2010:937, 938, 939). Gastric bleeding is also a complication of warfarin therapy and can result in intestinal obstruction. The use of aspirin with warfarin can increase the risk for gastric bleeding (Maimon *et al.,* 2006:212).

In 2009 Brodsky and colleagues identified another serious complication due to warfarin therapy. The data they collected showed that warfarin can cause acute kidney injury. They explained that warfarin therapy causes glomerular haemorrhage and the obstruction of renal tubules by red blood cell casts. This complication is especially serious in older patients with underlying chronic kidney injury (Brodsky *et al.,* 2009).

There are certain risk factors associated with bleeding. Abdelhafiz and Wheeldon (2007) compiled a list of these risk factors. They are the same for all bleeding types, namely major and minor bleeding. The list is as follows:

- Age
- Sex
- History of:
  - → ischemic cerebrovascular stroke

- → hypertension
- → diabetes mellitus
- → congestive heart failure
- → ischemic heart disease
- → myocardial infarction
- → peripheral vascular disease

- Number of co-morbidities
- Number of medications
- Mean INR
- Mean range of INR
- Mean frequency of INR check

A study done in 2003 (Essalihi *et al.,* 2003) showed that warfarin causes significant changes in the vascular system, which leads to isolated systolic hypertension. Although this study was only performed on rats, the authors claim that this can be modelled on humans. They claim that treatment with warfarin can lead to aortic medial calcification and that the pulse pressure can be increased, which together with other factors can lead to an increased systolic blood pressure. The amount of collagen in the blood vessels also increases, but a decrease in elastin is noted. All these factors contribute to the stiffening of the aorta, and this eventually leads to isolated systolic hypertension (Essalihi *et al.,* 2003).

Skin necrosis induced by warfarin therapy is also a serious problem. Skin necrosis is a rare side-effect that only effects about 0.01% to 0.1% of patients on warfarin therapy. Until today only 200 cases have been reported. It is a serious condition as it can affect large or multiple areas of the body. It has been reported to occur on the abdomen, thighs, legs and breast tissue in young girls and women. Other smaller areas are also affected, including the arms, back and penis. More than one area on the body can be affected simultaneously. Patients most affected by this condition are middle aged women who underwent treatment for a pulmonary embolism or thrombophlebitis. Changes in the skin can occur around 24 hours after treatment was initiated. Skin necrosis appears 3 to 10 days after treatment was initiated (Nazarian *et al.,* 2009:327).

Skin necrosis induced by warfarin therapy is usually due to a paradoxical hypercoagulable state that is caused by the inhibition of some vitamin K-dependent factors. This causes an inbalance in the procoagulant and anticoagulant pathway. A risk factor for this condition is a deficiency of protein C. Other factors that also play a role are protein S deficiency,

antithrombin III deficiency, factor V Leiden mutation, or the antiphospholipid antibody syndrome. The clinical presentation of skin necrosis induced by warfarin therapy is very similar to the clinical presentation of other skin diseases. These diseases include calciphylaxis, micro-embolisation, heparin-induced skin necrosis, disseminated intravascular coagulation, pupura fulminans, necrotizing faciitis, cryoglobulinemia, inflammatory breast cancer, decubitus ulcers and finally snake venom-induced skin necrosis (Nazarian *et al.,* 2009:328).

The treatment of this condition usually necessitates early diagnosis and discontinuation of warfarin therapy. Otherwise treatment is supportive, for instance the administration of FFP and vitamin K which is necessary to restore protein C and S levels in the body. Other anticoagulants like heparin are then used. A topical antibiotic can also be used. In serious cases the treatment involves surgical debridement with or without skin grafting, mastectomy or amputation. This severe form of treatment is indicated in about 50% of cases (Nazarian *et al.,* 2009:331).

Another form of skin necrosis induced by warfarin therapy is a condition called purple-digit syndrome. Purple-digit syndrome occurs on the tips of the fingers or toes and affects more men than women. As with any other warfarin-induced skin necrosis this condition presents a few days after warfarin therapy has been initiated. There are a few differences though. It can also first occur a few weeks after the initiation of warfarin therapy and can continue after warfarin therapy has been withheld. Researchers speculate that cholesterol emboli also play a role in this condition. Purple-digit syndrome requires surgical intervention, debridement and skin grafting, and amputation. Death can also occur if the physician fails to recognise and diagnose this skin condition (Egred & Rodrigues, 2005:294, 295).

Penile ischemia is also caused by warfarin-induced skin necrosis. It can lead to amputation, restructuring, and penile skin grafting (Talbert & Wood, 2011:335).

Cases of dermatotoxicity have also been reported among users of rodenticides. The skin is a point of entry for hydroxycoumarins. Warfarin toxicity has been reported in patients who handle warfarin. Warfarin tends to "linger" in the skin for up to 72 hours, which leads to changes in the skin's histopathology and eventually skin necroses. Researchers noted an increase in the levels of malondialdehyde (an end-product of lipid peroxidation), which indicates that oxidative activity took place. This is how researchers discovered that dermatotoxicity occurred in warfarin-treated skin (Kataranovski *et al.,* 2005:207, 214).

There are other side-effects of warfarin therapy that are not associated with haemorrhagic disorders. In 2002, Simon *et al.* studied the effects long-term warfarin therapy have on bone

density. They performed the study on rats and the following results were obtained after testing the bone strength of the rats:

- there was a time- and dose-dependent decrease in femoral bone strength
- cancellous bone volume decreased
- osteoblast number and activity decreased
- the number of osteoclast increased (Simon *et al.,* 2002:356)

All of these factors result in a decrease in bone strength. The researchers speculate that the reason for this is that bone also contains Vitamin K reductase enzymes. They state that osteoblasts contain vitamin K reductase and vitamin K epoxide reductase. These enzymes perform a similar function to the vitamin reductase enzymes found in the liver. They are also responsible for the formation of γ-carboxyglutamic acid proteins like the vitamin K reductase enzymes in the liver. These proteins include osteocalcin, matrix GLA protein and protein S (Kataranovski *et al.,* 2005:207; Rejnmark *et al.,* 2007:338; Simon *et al.,* 2002:357). Researchers also noted that a decrease in vitamin K resulted in increased levels of osteocalcin that was undercarboxylated. High levels of this form of osteocalcin coupled with low plasma levels of phylloquinone results in a decrease of bone mineral density, and thus an increase in the risk of bone fracture (Rejnmark *et al.,* 2007:338). It is obvious that normal bone turnover is affected by the inhibition of the formation of these proteins by warfarin (Simon *et al.,* 2002:357).

Warfarin is also teratogenic. The use of warfarin during pregnancy can cause a number of problems, most of them developmental defects. The major complication that can occur from use during pregnancy is a condition called warfarin embryopathy (Blickstein & Blickstein, 2002:222; Kataranovski *et al.,* 2005:207). It consists of a number of defects that can occur, including nasal hypoplasia, stippled epiphyses and distal extremity hypoplasia (Simon *et al.,* 2002:353). These conditions can be due to the effect warfarin has on the vitamin K reductase enzymes. Warfarin has an effect on the embroyonic calcium deposition, which has an effect on the formation and growth of bone. This whole process occurs in the first six to nine weeks of pregnancy (Blickstein & Blickstein, 2002:222; McLintock, 2011:S57). Stippled calcification, extremity shortening, vertebral abnormalities and nasal hypoplasia can result from this effect warfarin has on the vitamin K reductase enzymes (Blickstein & Blickstein, 2002:222). Neurological abnormalities that can occur are ventral midline dysplasia with corpus callosum agenesis and dorsal midline dysplasia with optic atrophy, and Dandy-Walker malformation (McLintock, 2011:S57). Other serious consequences of warfarin therapy during pregnancy are abortion, foetal or neonatal haemorrhage and ultimately

intrauterine death (Brunton, 2012). Many of these defects can occur in the first trimester of pregnancy. However, physicians do prescribe warfarin therapy in the second or third trimester of pregnancy, but the risk of central nervous system abnormalities is still present. Generally in the United States warfarin therapy during pregnancy is completely contra-indicated (Brunton, 2012; Marks, 2007:228).

Other side-effects that can occur with warfarin therapy are hepatitis, priapism, alopecia, urticaria, dermatitis, fever, nausea, diarrhoea, abdominal cramps, and finally anorexia. These side-effects are uncommon (Brunton, 2012; Nazarian *et al.,* 2009:325).

2.2.5.3 Dosage and monitoring

Warfarin is one of the most widely used anticoagulants to date (Limdi *et al.,* 2004:118). Jonas and McLeod (2009) estimate that roughly 20 million prescriptions for warfarin are written in the United States annually. This is alarming since warfarin is one of the most difficult drugs to prescribe and monitor (Jonas & McLeod, 2009:375). It is a dangerous drug because it has a narrow therapeutic index, consequently over- and under dosing is a great possibility, and this may easily lead to haemorrhage or thrombosis depending on which end of the scale is tipped (Asnis *et al.,* 2007:213; Cao *et al.,* 2007:175; Jonas & McLeod, 2009:375; Limdi *et al.,* 2004:118). Another reason why warfarin therapy is so difficult to manage is that there are numerous factors that influence the pharmacological activity of this drug. These factors cause a variable biological response that translates into a great deal of inter-patient variation in the effects of warfarin (Ansell *et al.,* 2005:37; Wells *et al.,* 2010:e259). These factors include age, body mass index, and nutritional status of the patient, co-morbid disease sates, co-prescribed medications, pharmacogenetics, and finally, ethnicity (Asnis *et al.,* 2007:213; White, 2010:194-200). These factors will be discussed later.

The first test that was used to measure the effect of warfarin was the prothrombin time (PT). The PT reacts to a decline in clotting factors dependent on vitamin K and this decline is due to the reduction of these clotting factors by warfarin. These clotting factors include factors II, VII and X. As the clotting factors decline, the PT is prolonged. The PT test is performed by using thromboplastin. However, the PT can be very inaccurate in predicting the effect of warfarin because there are variations in the response of thromboplastin to warfarin. It is because of this shortcoming that the INR was developed (Hirsh *et al.,* 2003:636; Motykie *et al.,* 1999:988). The INR is calculated using the PT and the testing laboratory's unique thromboplastin reagent lot. This value is then compared with the international standardised value determined by the World Health Organization (Leiker *et al.,* 2009:227; Motykie *et al.,* 1999:988).

Another method of analysis was developed in order to evaluate the quality of warfarin treatment while using the INR. The TTR (time in therapeutic range) was created to describe the time in which the patient's INR is actually in the therapeutic range (Nieuwlaat *et al.,* 2010:e128). Different disease states require different INR ranges for therapy to be effective. To give an example, patients suffering from atrial fibrillation require their INR to be in a range of 2 to 3, or even as low as 1.5, depending on the disease state (Habib *et al.,* 2008:129; Hirsh *et al.,* 2003:1641; Nieuwlaat *et al.,* 2010:e128). If the INR falls below a value of 2, the chance of stroke can increase. If the INR rises to a value above 3, the chance for a bleeding complication can increase (Nieuwlaat *et al.,* 2010:e128; Wright & Dufull, 2011:1100, 1101). Other disease states such as patients with mechanical heart valves or other conditions that may increase the risk of thrombosis, require their INR to be in a range of 2-3.5 (Hirsh *et al.,* 2003:1641; Katzung *et al.,* 2009:596).

Warfarin dosing can be divided into two phases, namely the initial phase (when warfarin therapy is started for the first time) and the maintenance phase (when warfarin therapy is prescribed for chronic use). In the initial phase of warfarin therapy the INR must be tested repeatedly until a stable relationship between the dose and the response to warfarin is achieved (Asnis *et al.,* 2007:213; Hirsh *et al.,* 2003:1637). It is only then that INR testing can occur less frequently. After the initiation of oral warfarin therapy, an anticoagulant effect can be expected within 2-7 days. Usually warfarin therapy is started with a dose of 5 mg per day (Hirsh *et al.,* 2003:1637).

Even though warfarin therapy is initiated at a dose of 5 mg per day, it is much more complicated to predict the maintenance dose to achieve and maintain the target INR. The maintenance dose can range from 0.5 mg to 60 mg per day depending on the patient factors that may influence the warfarin dose (Cao *et al.,* 2007175; Wright & Dufull, 2011:1100). Therefore, frequent INR testing is crucial. Adjusting the dose according to the INR has proven to be very difficult and time consuming. Medical practitioners struggle daily with the "how's, where's and when's" of warfarin dose adjustment. As a result, several dose adjustment strategies have been developed (Cao *et al.,* 2007:175). These dose adjustment strategies will be discussed briefly.

The first strategy that will be discussed is the warfarin nomograms. In an effort to standardise warfarin dosing and to achieve the desired INR in a short period of time, the warfarin nomogram was developed. These nomograms resulted in patients having to test their INR less frequently in the initial stages of warfarin therapy. They are also very helpful in the hospital setting as it saves time and hospital staff can easily work out the correct dose for a patient even if they did not initiate treatment (which is common during hospital staff shift

changes) (Asnis *et al.*, 2007: 213, 216; Monkman *et al.*, 2009:275, 278). Tables 2.10 and 2.11 are examples of such nomograms respectively adapted from Asnis *et al.* (2007) and the Warfarin Dosing Nomogram for Maintenance Therapy developed by the University of Washington Medical Centre Anticoagulation Services June 2010.

Table 2.11: An example of a warfarin dosing nomogram

International Normalised Ratio																	
DAY	**1.0**	**1.1**	**1.2**	**1.3**	**1.4**	**1.5**	**1.6**	**1.7**	**1.8**	**1.9**	**2.0**	**2.1**	**2.2**	**2.3**	**2.4**	**2.5**	**2.6**
0	5	5	5	2.5	2	0	0	0	0	0	0	0	0	0	0	0	0
+1	5	5	5	3	2	1	0	0	0	0	0	0	0	0	0	0	0
+2	7.5	6	5	5	4	3	2	1	1	0	0	0	0	0	0	0	0
+3	7.5	7	6	5	5	4	3	2.5	2	1	1	0	0	0	0	0	0
+4	8	7.5	7	6	5	5	4	3	2.5	2	2	1	1	0	0	0	0
+5	10	7.5	7.5	6.5	6	5	5	4	3	2.5	2	1.5	1.5	1	0.5	0	0
+6	10	7.5	7.5	6.5	6	5	5	4	3	2.5	2	2	1.5	1	1	1	0
+7	10	7.5	7.5	5	5	5	5	4.5	3	2.5	2.5	2	1.5	1	1	0.5	0
>+8	10	7.5	7.5	5	5	5	4.5	4	3	2.5	2	2	2	1	1	0.5	0

Table 2.12: An example of a warfarin dosing nomogram and suggested dose adjustments to achieve the desired INR

For goal INR 2-3	Required dosing adjustments	For goal INR 2.5-3.5
INR <1.5	• A booster must be considered that is 1.5-2 times the daily maintenance dose • Continuation of the previous maintenance dose must be considered if the decline in the INR is caused by a temporary factor (e.g. if a dose was missed) • The maintenance dose must be increased by 10-20% if a dose adjustment is needed	**INR <2.0**
INR 1.5-1.8	• A booster must be considered that is 1.5-2 times the daily maintenance dose • Continuation of the previous maintenance dose must be considered if the decline in the INR is caused by a temporary factor (e.g. if a dose was missed) • The maintenance dose must be increased by 5-15% if a dose adjustment is needed	**INR 2.0-2.3**
INR 1.5-1.9	• No dose adjustments may be necessary if: - the last 2 INRs were in range - there is no clear explanation why the INR is out of range - the INR does not represent an elevated risk for thrombo-embolism • A booster must be considered that is 1.5-2 times the daily maintenance dose • Continuation of the previous maintenance dose must be considered if the decline in the INR is caused by a temporary factor (e.g. if a dose was missed) • The maintenance dose must be increased by 5-10% if a dose adjustment is needed	**INR 2.3-2.4**

Table 2.11 (continued): An example of a warfarin dosing nomogram and suggested dose adjustments to achieve the desired INR

INR 2.0-3.0	The desired range	INR 2.5-3.5
INR 3.1-3.2	• No dose adjustments may be necessary if: - the last 2 INRs were in range - there is no clear explanation why the INR is out of range - the INR does not represent an elevated risk for thrombo-embolism • Continuation of the previous maintenance dose must be considered if the decline in the INR is caused by a temporary factor (e.g. acute alcohol consumption) • The maintenance dose must be increased by 5-10% if a dose adjustment is needed	INR 3.6-3.7
INR 3.3-3.4	• Withholding 0.5-1 dose must be considered • Continuation of the previous maintenance dose must be considered if the decline in the INR is caused by a temporary factor (e.g. acute alcohol consumption) • The maintenance dose must be increased by 5-10% if a dose adjustment is needed	INR 3.8-3.9
INR 3.5-3.9	• Withholding 1 dose must be considered • Continuation of the previous maintenance dose must be considered if the decline in the INR is caused by a temporary factor (e.g. acute alcohol consumption) • The maintenance dose must be increased by 5-15% if a dose adjustment is needed	INR 4.0-4.4
INR ≥ 4.0	• Withhold therapy until the INR is smaller than the upper limit of the therapeutic range • The use of a small dose of vitamin K must be considered • Continuation of the previous maintenance dose must be considered if the decline in the INR is caused by a temporary factor (e.g. acute alcohol consumption) • The maintenance dose must be increased by 5-15% if a dose adjustment is needed	INR ≥ 4.5

The second strategy that will be discussed is the use of computer models that are based on the Bayesian forecasting model that aids in the initiation and maintenance of warfarin therapy. The Bayesian model incorporates the expected response an individual patient has to a drug. The individual drug response is based on pharmacokinetic and pharmacodynamic models and parameters in which the values are derived from a previous population. The response data are from an individual patient (Wright & Dufull, 2011:1101). There are numerous advantages when using such a computer model. These advantages are as follows:

- the patient can be discharged from hospital earlier;
- the patient does not have to be monitored as frequently;
- it is a quicker way of predicting the dose;
- different INR responses can be calculated for different doses before the dose is administered to the patient;

- medical practitioners can "double-check" their dose calculations;
- other hospital staff such as nurses can help with patient monitoring without the supervision of a general practitioner or specialist (Motykie *et al.,* 1999:992).

Variables that influence warfarin dosing include age, body mass index, and nutritional status of the patient, co-morbid disease sates, co-prescribed medications, pharmacogenetics, and finally, ethnicity (Asnis *et al.,* 2007:213; White, 2010:194-200). Genetic factors play an important role in predicting the correct warfarin dose. The pharmacogenetics of warfarin dosing was discussed thoroughly in section 2.2.4.5. In summary, the genes encoding CYP2C9 and VKORC1 present with the most widely known genetic polymorphisms. The *S*-enantiomer of warfarin is mainly catalysed by the CYP2C9 enzyme to its inactive metabolites. Several polymorphisms of this enzyme exists, such as CYP2C9*2 and CYP2C9*3. These two polymorphisms play a major role in the variability that exists in warfarin dosing. These two polymorphisms usually result in the patient requiring a lower warfarin dose. Patients presenting with polymorphisms in the VKORC1 enzyme also requires a lower warfarin dose (Cho *et al.,* 2011:1372, 1374; Wells *et al.,* 2010:e259).

Research done on warfarin pharmacogenetics encourages the development of dosing algorithms that incorporate the genotype information of the patient. These algorithms include clinical and genetic factors that may influence warfarin doses. Studies done on these algorithms have shown that they accurately predict warfarin doses (Cho *et al.,* 2011:1374, 1377; Finkelman *et al.,* 2011:612). An example of dosing algorithms can be found in section 2.2.4.5.

The clinical factors that influence warfarin dosing will now be discussed shortly.

Age is a major factor that influences warfarin dosing. As patients get older, their sensitivity to warfarin increases, so their dose requirements decrease. A number of factors can contribute to this. As a patient gets older their dietary intake of vitamin K can decrease, leading to a declining production of clotting factors. It is therefore important to note that the normal initiation dose of 5mg per day may lead to over-anticoagulation in older patients. Consequently attention should be paid to the fact that older patients may need a lower warfarin initiation dose (Jonas & McLeod, 2009:383; White, 2010:194, 195).

The Body Mass Index (BMI) of a patient may also be considered a factor influencing warfarin dosing. A clear relationship between BMI and warfarin dosing has not yet been established, but it can be theoretically argued that serum albumin levels can change with a change in BMI, thus warfarin dosing requirements can also change (White, 2010:195).

The nutritional status of a patient can have a major effect on warfarin dosing. Variations in vitamin K intake can cause alterations in a patients' INR. Fluctuations in vitamin K intake can be due to a number of factors (Jonas & McLeod, 2009:383; White, 2010:195). These factors include:

- malnutrition;
- chronic illness, which causes a decreased dietary intake of vitamin K;
- parenteral nutrition over a long period of time;
- alterations in gastrointestinal flora due to administration of antibiotics;
- syndromes that cause fluctuations in fat absorption.

These patients usually require a lower warfarin dose. There are some dietary factors that can cause a patient to require a higher dose. These are usually patients on a high-protein, low-carbohydrate diet. Acute consumption of alcohol may also cause fluctuations in a patient's INR (Jonas & McLeod, 2009:383; White, 2010:195).

Various chronic illnesses can result in altered warfarin dosing requirements. Patients with liver disease may need a lower dose due to a decline in the production of several clotting factors by the liver. The employment of vitamin K by the liver may be altered when the liver is diseased. Inevitably, the metabolism of warfarin can be decreased. Patients suffering from kidney failure also require a lower dose. It has been found that these patients experience fluctuations in their INRs. These patients therefore require an average daily dose in the range of 3.9-4.8 mg. Thyroid diseases also cause difficulties in dosing as patients with hyperthyroidism are more sensitive to the effects of warfarin and therefore need a lower dose. Patients suffering from hypothyroidism may need higher warfarin doses, but as their thyroid function is restored, their doses need to be reduced (White, 2010:196, 197).

Drug interactions are probably the biggest contributor to fluctuations in warfarin dosing. This factor will be discussed thoroughly in the next section.

All of these factors mentioned above contribute to the difficulty in warfarin therapy and management. In an ideal world an anticoagulant will exist with predictable pharmacokinetics, a wide therapeutic window, little to no drug interactions and no side-effects, unfortunately this is not an ideal world (Ansell, 2005:135; Limdi *et al.,* 2004:119). For years the INR has been used in the hope that it will provide some form of standard when it comes to reporting the results of therapy. Even though the INR is successful in the reporting of its values, it still does not completely help with the complexity of warfarin therapy. Even if the INR is in its therapeutic range, it is still difficult to keep it in the therapeutic range because all of the

factors discussed above continuously influence the patients' response to warfarin. It is therefore critical that the doses are adjusted accordingly, but this has also been proved to be difficult (Ansell, 2005:135).

In 2004, Limdi *et al.* did a study on patients' INRs. During the course of one year the research team recorded the INRs of 576 patients. They found that the INRs of 316 (55%) patients were outside of the desired therapeutic range. In 169 (29%) patients a change in dose was required. Lastly, the researchers found that in 147 (26%) patients an intervention was required (Limdi *et al.*, 2004:119). It is therefore clear that the monitoring of patients on warfarin therapy is important. According to Nieuwlaat *et al.* for warfarin therapy to be effective, the patient's TTR should be 60-70%. If a patient's TTR is below 40%, then warfarin therapy is not effective and the patient can be in harm's way (Hirsh *et al.*, 2003:1637; Nieuwlaat *et al.*, 2010:e128).

In order to monitor the TTR effectively, the INR must be tested frequently. Woods *et al.* recommend that the INR should be tested every two to four weeks to keep the INR in a range of 2.0-3.5. INR testing usually requires a blood test where venous blood is drawn. The time taken to draw the blood, deliver it to the lab and to analyse the blood can be extensive and an inconvenience to the patient, and therefore the patient will be less likely to come for frequent INR testing (Woods *et al.*, 2004:162).

Several methods have been developed to improve the monitoring of warfarin therapy. These methods will now be discussed briefly.

In an effort to simplify the monitoring of warfarin therapy several point-of-care (POC) systems have been developed. These systems comprise the use of anticoagulation meters to measure a patient's INR. These meters only require a drop of blood from a finger prick to determine the INR. This paved the way for INR monitoring at home (Hirsh *et al.*, 2003:1637; Woods *et al.*, 2004:162).

Patient self-testing (PST) is the practice of establishing one's own INR, but the dose adjustments are still made by a medical practitioner. Patient self-management (PSM) is the practice where the INR testing and the dose adjustments are made by the patient within certain parameters. The medical practitioner is less involved in this case (Ansell *et al.*, 2005:38; Ansell & Hughes, 1996:1098-1099). By using these management tools, control over anticoagulation can be improved. Patients are now able to test their INRs more frequently without disturbing their normal routine. Ryan *et al.* lists a few advantages of PST and PSM. They include:

- fewer regular out-patient visits;
- less venepunctures;
- decreased travel expenses;
- less time off work (Ryan *et al.,* 2010:e345).

PST and PSM allows for more regular INR testing, which leads to more INRs being in the required therapeutic range. Regular INR testing is dependent on a number of factors including "patient stability, compliance, fluctuations in co-morbid conditions, the addition or discontinuation of other medications, changes in diet, the quality of dose-adjustment decisions and the stage of treatment" (Ansell *et al.,* 2005:41). Despite all of these factors, a great deal of satisfactory anticoagulation can be achieved (Hirsh *et al.,* 2003:1639). An important result of PST and PSM is the awareness that it creates. Patients are empowered by the information that they get from PST and PSM. Awareness is increased about the various factors that may influence the patients' INR. Better control over anticoagulation can result from this (Ryan *et al.,* 2010:e348).

Patients that do not want or cannot monitor their warfarin therapy at home can still go to anticoagulation clinics. These clinics' primary focus is the management of patients on anticoagulation therapy (Ansell & Hughes, 1996:1095). Most of these clinics also use the POC systems; therefore these patients experience less pain during testing as no venous blood is used. Clinic visits are also shorter because the testing process is quicker (Woods *et al.,* 2004:164). There are still a few disadvantages when it comes to anticoagulation clinics. Patients may be required to visit the clinic eight to 12 times a year, and this may result in unnecessary costs such as travel expenses and "out-of-pocket expenses" (Jowett *et al.,* 2008:207). Even with these disadvantages POC systems, whether done at home or at a clinic, is still very successful. Patients using the POC systems showed to have fewer dose changes, are more empowered by this form of therapy and therefore had better compliance, and more patients' INRs were in the target therapeutic range (Ansell & Hughes, 1996:1099).

Monitoring warfarin therapy in children is very different from that in adults. There are some additional factors in children that influence their response to warfarin. Children also need frequent INR monitoring, since they have variable nutritional intake, greater use of co-prescribed medications, and are more susceptible to colds and flu. All of these factors may influence the metabolism of warfarin. The use of POC systems is critical in children. Drawing venous blood can be challenging as many children are afraid of needles and locating a suitable vein for venepuncture can be difficult (Bauman *et al.,* 2009:707; Bauman *et al.,* 2010:e110). Adherence can be a problem due to the fact that when a child needs to go for

INR testing, he/she may have to leave school in the middle of the day and the parents may have to leave work (Bauman *et al.*, 2010:e110).

Usually children that are required to go on warfarin therapy are those with heart disease. Some of the indications for warfarin in children are the same as those in adults such as treatment for deep vein thrombosis, primary thromboprophylaxis or secondary thromboprophylaxis. The latter of the three are usually required in children who have undergone the Fontan procedure (Bauman *et al.*, 2010:e110; Streif *et al.*, 1999:3007).

The loading and maintenance doses in children can differ greatly from those in adults. In children the warfarin dose is closely related to the child's weight. As the child's weight increases, so does the dose (in mg per day) (Tait *et al.*, 1996:230). Streif *et al.* (1999) argues that the dose in children must be calculated using the body surface area of the child rather than the weight as this is more accurate (Streif *et al.*, 1999:3012). Tait *et al.* (1996) found that normally the loading dose of warfarin for children is usually around 0.2 mg/kg/day (Tait *et al.*, 1996:230). Other studies show that a loading dose of 0.3 or 0.4 mg/kg is required (Streif *et al.*, 1999:3013). However, the maintenance dose is different. They found that children under the age of one year may need a dose of 0.32 mg/kg/day to sustain an INR of 2-3 (Tait *et al.*, 1996:230). Streif *et al.* (1999) state that a maintenance dose of 0.16 mg/kg is necessary to maintain the same INR range. Generally children under the age of two years require a maintenance dose that is double that of children ages 11-18 years and triple that of adults (Tait *et al.*, 1996:230).

Streif *et al.* (1999) noticed a few differences in warfarin management between children ≤ 1-6 years of age compared to adults on warfarin therapy. It was found that these children need:

- higher warfarin doses;
- increased overlap time with heparin;
- more time to reach the therapeutic INR;
- increased frequency of INR testing;
- increased frequency of dose adjustments (Streif *et al.*, 1999:3012).

Another problem noticed with children is that those who receive enteral feedings require higher warfarin doses than those who do not (Streif *et al.*, 1999:3012). Dickerson (2008) speculates that this is due to an interaction of warfarin with the vitamin K in the enteral feeding formulation. He recommends that enteral feeding should be suspended one hour before and after warfarin administration. The rate of enteral feeding should also be increased from 24 hours per day to 22 hours per day because feeding is disrupted (Dickerson,

2008:1048, 1051). Warfarin resistance is also experienced in children with short-bowel syndrome due to decreased warfarin absorption (Streif *et al.*, 1999:3012).

It is clearly illustrated above that the clinical course for warfarin therapy in children can be more challenging than that of adults (Streif *et al.*, 1999:3013).

Anticoagulation management in the elderly can also be challenging. There are several factors that come with increasing age affect warfarin dosing. These factors include:

- lower levels of albumin in the body, which results in a lower volume of distribution;
- intake of lower levels of vitamin K resulting in a decline in the production of coagulation factors;
- decreased absorption of vitamin K;
- polypharmacy, which increases the potential of warfarin drug-drug interactions;
- decreased drug metabolism in the liver (Garcia *et al.*, 2005:2053).

The problem with warfarin dosing in the elderly is that many warfarin initiation nomograms are based on a younger population and do not account for the factors mentioned above (Garcia *et al.*, 2005:2050). Dosing algorithms pose the same problems as they are also based on a younger population. Schwartz *et al.* (2011) found that these algorithms overestimated doses for elderly patients and do not identify older patients requiring lower warfarin doses (Schwartz *et al.*, 2011). Over-anticoagulation and inadequate estimation of the maintenance dose may result from this. Due to the factors mentioned above, elderly patients require lower warfarin doses (Gedge *et al.*, 2000:31). It is therefore important to note that the recommended starting dose for warfarin of 5mg per day may be excessive in the elderly and may result in supratherapeutic INR levels (Garcia *et al.*, 2005:2050).

Another difficulty that may arise with warfarin therapy in the elderly is the fact that these patients also require frequent INR testing. Most elderly patients rely on other people for transportation and this can pose a problem. Many may also be limited in a physical sense and may therefore be less mobile (Garcia *et al.*, 2005:2050).

Medical practitioners may be hesitant to prescribe warfarin to elderly patients. The risk for bleeding and intracranial haemorrhage increases with age. Medical practitioners are more mindful when prescribing warfarin to these patients, as these patients have a higher tendency to fall (Capodanno & Angiolillo, 2010:1688).

In elderly patients there is also a difference between the required warfarin doses in different age groups and differences in doses between men and women (Garcia *et al.*, 2005:2053).

Table 2.12 shows these differences in doses between age groups and that of males and females as adapted from Garcia *et al.* (2005).

Table 2.13: The difference in the daily dose of warfarin between age groups and between males and females in an elderly population

	MALE	FEMALE
Age (yrs)	Warfarin dose (mg)*	Warfarin dose (mg)*
50-59	(4.0, 6.4)	(3.9, 6.0)
60-69	(3.6, 5.7)	(2.9, 5.4)
70-79	(3.2, 5.4)	(2.5, 4.6)
80-89	(2.5, 5.0)	(2.5, 4.3)
≥90	(2.6, 4.0)	(2.0, 3.6)

**Values expressed as the 25th percentile and the 75th percentile of the daily warfarin dose*

2.2.6 INTERACTIONS

This section will feature a brief discussion on what drug-drug interactions are, the different mechanisms of drug interactions and the different ratings assigned to drug interactions.

2.2.6.1 Introduction

There are a number of factors that can have an effect on the pharmacological action of a drug. These factors include other drugs, food and nutritional supplements (Delafuente, 2003:134). A few definitions for drug-drug interactions follow below, since the working definitions vary (Tatro, 2011:xvii). Examining different definitions will enable a better understanding of drug-drug interactions.

A drug-drug interaction can be:

> "The phenomenon that occurs when the effects or pharmacokinetics of a drug are altered by prior administration or co-administration of a second drug." (Tatro, 2011:xvii)

> "When the effects of one drug are changed by the presence of another drug, herbal medicine, food, drink or by some environmental chemical agent." (Baxter, 2012)

> "When the spheres of activity of two drugs overlap, so the action of one drug will affect the behaviour of another." (Corrie & Hardman, 2011:156)

"Unfavourable clinical events which are caused by abnormally increased or decreased drug concentrations in the body as consequence of co-administration of other drug(s)." (Hisaka *et al.*, 2010:231)

"Where one drug has the potential to interfere with the pharmacological actions of another drug." (Delafuente 2003:134)

Since 1985 the use of prescription drugs has increased considerably (Delafuente 2003:134). It is becoming common practice for patients to be treated with more than one drug. The use of over-the-counter medications, vitamins and herbal supplements are also on the rise. It is because of this that more attention should be given to potential drug interactions. As a result of drug interactions, the pharmacokinetics of the drug can be altered, and this can lead to altered rates of absorption, biotransformation, and excretion or altered protein binding. The pharmacodynamics of a drug can also be affected and this may lead to competition at receptors, and non-receptor pharmacodynamic interactions (Brunton, 2012). Figure 2.11 is a schematic representation of all the mechanisms of drug interactions that will be discussed in this section as adapted from Brunton (2012); Corrie and Hardman (2011); Delafuente (2003); Pleuvry (2005); Baxter (2012) and Tatro (2011). The most important interactions will be discussed thereafter.

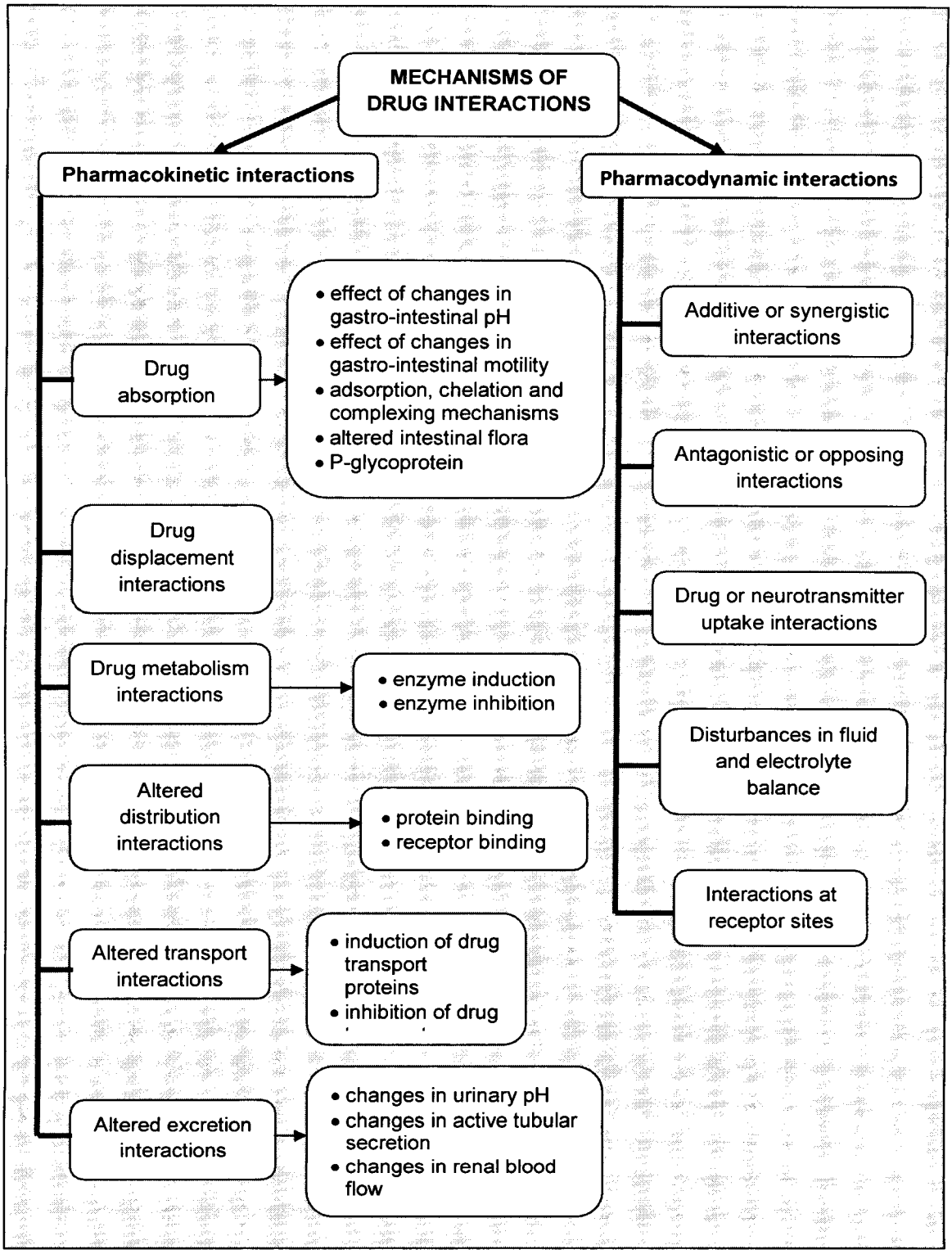


Figure 2.11: Schematic representation of the mechanisms of drug interactions

2.2.6.2 Mechanisms of drug interactions

Drug interactions are usually divided into two major groups, namely pharmacokinetic interactions and pharmacodynamic interactions.

Pharmacokinetic interactions are those in which the rate or extent of the absorption, distribution, elimination and excretion of a drug is altered by the presence of another drug (Pleuvry, 2005:129; Tatro, 2011: xvii). An altered rate of change of certain pharmacokinetic parameters is an indication of this. These parameters include peak serum concentration, area under the concentration-time curve, half-life, and total amount of drug excreted in the urine (Corrie & Hardman, 2011:156; Tatro, 2011: xvii).

With pharmacodynamic interactions the response of a patient to a drug is altered, but the pharmacokinetics are not altered. An altered drug response occurs between drugs with the same or opposite pharmacological effects. These interactions can occur at the same or different receptors (Corrie & Hardman, 2011:156; Pleuvry, 2005:130; Tatro, 2011: xvii).

2.2.6.3 Pharmacokinetic interactions

- **Drug absorption interactions**

Absorption interactions can occur when one drug changes the absorption traits of another drug (Delafuente, 2003:136). The most absorption interactions occur in the gastro-intestinal tract where oral drugs are absorbed (Tatro, 2011: xviii). The interactions that occur here most of the time lead to a reduced absorption of the drug. Two problems can occur. Either the rate of absorption or the total amount of drug absorbed can be altered. For oral drugs that are chronically administered the total amount of drug absorbed is essential for a drug such as warfarin. For oral drugs that are acutely administered (for example acetaminophen), the rate of absorption is essential. For effective therapy to be achieved, peak plasma concentrations are essential (Pleuvry, 2005:132; Baxter, 2012).

There are sites in the body other than the gastro-intestinal tract where absorption of drugs can be altered. Local blood flow will influence the rate of absorption at sites where drugs are administered subcutaneously or intramuscularly (Corrie & Hardman, 2011: 158).

(a) The effect of changes in gastro-intestinal pH

Changes in the pH of the gastro-intestinal tract may also influence absorption. Drugs that increase the gastro-intestinal pH will improve the absorption of basic drugs, for example ranitidine and triazolam (Baxter, 2012; Brunton, 2012).

(b) The effect of changes in gastro-intestinal motility

The principal location of absorption of drugs in the gastro-intestinal tract is the small intestine. Gastric emptying is therefore an important factor to consider in drug absorption interactions. Drugs that promote gastric emptying will increase drug absorption, for example a prokinetic agent (Corrie & Hardman, 2011:158; Pleuvry, 2005:132). Absorption can be unpredictable here as some may argue that prolonged exposure in the small intestine may promote metabolism of the drug by agents in the small intestine, and thus decrease the absorption of the drug (Baxter, 2012). Drugs that reduce gastric emptying will decrease drug absorption, for example a muscarinic receptor antagonist (Corrie & Hardman, 2011:158; Pleuvry, 2005:132).

(c) The effect of adsorption, chelation and complexing mechanisms

The absorption of tetracycline antibiotics are altered by dairy products as these antibiotics are chelated to the calcium ions in dairy products (Baxter, 2012; Delafuente, 2003:136). Antacids can therefore alter the absorption of a number of drugs due to adsorption. In addition, ion exchanges can occur between drugs, which causes adsorption to one another and therefore altered absorption (Baxter, 2012).

(d) The effect of altered intestinal flora

As discussed is section 2.2.5.1, vitamin K is necessary for the formation of prothrombin and other blood clotting factors (Watanabe *et al.*, 2010:115). Vitamin K is present in the human body in two forms, as phylloquinones (vitamin K_1) and as menaquinones (Vitamin K_2). Phylloquionones are obtained from the diet and menaqiunones are synthesised by some intestinal flora. Giuliano *et al.* (2010) has found a correlation between patients with increased INRs and the use of broad spectrum antibiotics. They speculate that bacterial flora that has been altered by certain disease states could cause fluctuations in warfarin dosing requirements (Giuliano *et al.*, 2010:13, 15).

(e) P-glycoprotein

P-glycoprotein is a transporter protein that is found in the lining of the intestine. The function of this protein is to actively transport drug molecules from the cells back into the lumen of the intestine. Once transported back, these drug molecules can be further metabolised by CYP450 enzymes found in the wall of the intestine, such as CYP3A4. The absorption of some drugs may be impaired this way (Baxter, 2012; Hansten & Horn, 2011: PM-34, PM-35; Tatro, 2011: xviii).

The activity of P-glycoprotein can be inhibited or increased, thus leading to potential drug interactions. Rifampicin induces the activity of P-glycoprotein, which causes an increased transportation of digoxin from the cells back into the lumen of the intestine. A decrease in the blood plasma levels of digoxin then follows. Verapamil on the other hand, inhibits the activity of P-glycoprotein and thus fewer drugs are transported back into the lumen of the intestine. Digoxin plasma levels are then increased (Baxter, 2012; Tatro, 2011: xviii).

- **Drug displacement interaction**

Some drugs are bound to plasma proteins in order to be distributed in the body. An equilibrium remains between drugs bound to plasma proteins and those that are not (Baxter, 2012). The unbound fraction of drugs is pharmacologically active and can cause clinical effects while those still bound to plasma proteins are inactive (Brunton, 2012; Delafuente, 2003:137). As the unbound fraction gets metabolised, the bound fraction are released from the plasma proteins and become pharmacologically active (Baxter, 2012). As stated in section 2.2.4.2, warfarin is about 97% bound to plasma proteins and the unbound fraction is pharmacologically active (Appadu, 2010:252; D'Andrea *et al.*, 2008:128). Drug interactions can occur when drugs compete for binding sites on plasma proteins, which can cause one drug to be displaced by another (Corrie & Hardman, 2011:158). An example of such an interaction is the displacement of warfarin from plasma proteins by aspirin. The unbound fraction of warfarin in the blood plasma is increased and this may result in an enhanced pharmacological effect, which may lead to an increased risk for haemorrhage (Delafuente, 2003:137).

- **Drug metabolism interaction**

The main sites of metabolism of drugs are the liver, but metabolism of drugs can occur at other sites of the body such as the gastro-intestinal tract, skin, lungs, kidney, blood and placenta (Hansten & Horn, 2011:PM-18, PM-19) . There are two phases of metabolism, namely Phase I and Phase II metabolism. Phase I metabolism involves the cytochrome P450 enzymes, which transforms active molecules to inactive metabolites. Phase II metabolism usually occurs after Phase I metabolism and involved the attachment of a functional group to the molecule to make it water soluble so that it can be excreted by the kidneys (Hansten & Horn, 2011:PM-18, PM-19; Tatro, 2011: xx).

According to Delafuente (2003), the most important pharmacokinetic drug-drug interactions occur when one drug influences the metabolism of another drug (Delafuente, 2003:137). Phase I metabolism mainly occurs in the endoplasmic reticulum of liver cells. Phase I metabolism reactions are largely catalyzed by CYP450 enzymes, which constitute a large

family of different isoforms (Corrie & Hardman, 2011:158). The most important isoforms that will be discussed are those involved in the metabolism of warfarin. These CYP450 isoforms include CYP2C8, CYP2C9, CYP2C10, CYO2C18, CYP2C19, CYP1A1, CYP1A2 and finally CYP3A4 (AHFS, 2011; Anon., 2011; Guo *et al.*, 2006; Kaminsky & Zhang, 1997:68, 69; Mahajan *et al.*, 2011:11; Miller *et al.*, 2008:2211; Suzuki *et al.*, 2008:1155; Yamazaki & Shimada, 1997:1197; Zuo *et al.*, 2010:305).

(a) Enzyme induction

Drug metabolism can be increased. An increased metabolism occurs when the enzymes of the liver are induced by other drugs. The effectiveness of a drug can be diminished as these enzymes are removed from the system more rapidly and thus serum concentrations of the drug are decreased (Pleuvry, 2005:132; Tatro, 2011: xxi). Refer to section 2.2.4.4 for a full discussion on the metabolism of warfarin. Table 2.13 illustrates all the drugs that are enzyme inducers of those enzymes involved in the metabolism of warfarin as adapted from Hansten and Horn (2011) and Tatro (2011).

Table 2.14: The enzymes invloved in the metabolism of warfarin and the drugs that induce these enzymes

CYP450 subtypes	1A2	2C8	2C9	2C18	2C19	3A4
Enzyme inducers	Barbiturates Carbamazepine Charbroiled food Cigarette smoke Omeprazole Phenobarbital Phenytoin Primidone Rifampicin	Phenobarbital Primidone Rifapentine	Aprepitant Barbiturates Bosentan Carbamazepine Ethanol Griseofulvin Phenobarbital Phenytoin Primidone Rifampicin Rifapentine	None	Artemisinin Barbiturates Phenobarbital Phenytoin Primidone Rifampicin St. John's Wort	Barbiturates Bexarotene Bosentan Carbamazepine Dexamethasone Efavirenz Etravirine Glucocorticoids Griseofulvin Macrolide AB Modafinil Nafcillin Nevirapine Oxcarbazepine Phenobarbital Phenylbutazone Phenytoin Prednisone Primidone Rifabutin Rifampicin Rifapentine St. John's Wort Sulfinpyrazone

(b) Enzyme inhibition

In the same way that drug metabolism can be increased, it can be decreased. Drugs that decrease the rate of activity of the CYP450 enzymes can decrease the metabolism of other drugs. The result of this is a rise in the serum concentration of a drug that can be potentially harmful as drugs that have a narrow therapeutic index can easily become toxic (Pleuvry, 2005:132, Tatro, 2011: xxv). Table 2.14 illustrates all the drugs that are enzyme inhibitors of those enzymes involved in the metabolism of warfarin as adapted from Delafuente (2003), Hansten and Horn (2011) and Tatro (2011).

Table 2.15: The enzymes involved inthe metabolism of warfarin and the drugs that inhibit these enzymes

CYP450 subtypes	1A2	2C8	2C9	2C18	2C19	3A4
Enzyme inhibitors	Anastrozole	Anastrozole	Alcohol	Cimetidine	Chloramphenicol	Amiodarone
	Artemisinin	Omeprazole	Amiodarone		Cimetidine	Amprenavir
	Atazanavir		Anastrozole		Citalopram	Anastrozole
	Cimetidine		Capecitabine		Delavirdine	Aprepitant
	Ciprofloxacin		Chloramphenicol		Efavirenz	Atazanavir
	Citalopram		Cimetidine		Esomeprazole	Basiliximab
	Enoxacin		Clopidogrel		Etravirine	Chloramphenicol
	Erythromycin		Delavirdine		Felbamate	Cimetidine
	Ethinyl estradiol		Diclofenac		Fluoxetine	Clarithromycin
	Fluvoxamine		Disulfiram		Fluvoxamine	Clotrimazole
	Grapefruit juice		Efavirenz		Isoniazid	Conivaptan
	Mexiletine		Etravirine		Ketoconazole	Cyclosporine
	Mirtazapine		Fluconazole		Moclobemide	Danazol
	Norfloxacin		Fluorouracil		Modafinil	Darunavir
	Propranolol		Fluoxetine		Omeprazole	Dasatinib
	Ritonavir		Flurbiprofen		Oxcarbazepine	Diltiazem
	Sildenafil		Fluvastatin		Ritonavir	Delavirdine
	Tacrine		Fluvoxamine		Sildenafil	Dronedarone
	Zafirlukast		Gemfibrocil		Telmisartan	Erythromycin
	Zileuton		Imatinib		Ticlopidine	Ethinyl estradiol
			Ketoprofen		Tolbutamide	Fosamprenavir
			Leflunomide		Topiramate	Fluconazole
			Metronidazole		Tranylcypromine	Fluoxetine
			Miconazole		Troglitazone	Fluvoxamine
			Modafinil		Voriconazole	Grapefruit juice
			Omeprazole			Imatinib
			Phenylbutazone			Indinavir
			Phenytoin			Isoniazid
			Ritonavir			Itraconazole
			Sildenafil			Ketoconazole
			Sulfadiazine			Lapatinib
			Sulfamethizole			Miconazole
			Sulfamethoxazole			Mifepristone
			Sulfinpyrazone			Mirtazapine
			Sulfonamides			Nefazodone
			Trimethoprim			Nelfinavir
			Troglitazone			Nevirapine
			Valproic acid			Nicardipine
			Voriconazole			Nilotinib
			Zafirlukast			Norfloxacin
						Norfluoxetine
						Paroxetine

Table 2.14 (continued): The enzymes involved in the metabolism of warfarin and the drugs that inhibit these enzymes

CYP450 subtypes	1A2	2C8	2C9	2C18	2C19	3A4
Enzyme inhibitors						Mifepristone Mirtazapine Nefazodone Nelfinavir Nevirapine Nicardipine Nilotinib Norfloxacin Norfluoxetine Paroxetine Posaconazole Pazopanib Propoxyphene Propranolol Quinidine Quinine Quinupristin Ranitidine Ranolazine Ritonavir Saquinavir Sertraline Sildenafil Tamoxifen Telithromycin

- **Altered distribution interactions**

After drugs have been administered orally, it is absorbed and distributed in the body by the circulatory system. Some drugs are completely dissolved in the blood plasma or their molecules are transported in solution. Some, however, are bound to plasma proteins such as albumin (Baxter, 2012).

(a) Protein binding

Drugs that are highly bound to plasma proteins have the potential to be displaced by other drugs that are also highly bound. As discussed before, drugs bound to plasma proteins are inactive while the free fraction of drug is pharmacologically active. As drugs are displaced by

plasma proteins, the fraction of the unbound drug increases and this may cause the pharmacological activity of the drug to increase as well (Tatro, 2011: xx).

(b) Receptor binding

Drugs are ultimately distributed to receptor binding sites where they bind to receptors. Drug interactions can occur here as drugs can be displaced from receptor binding sites by other drugs. Digoxin can for example be displaced from its binding site in skeletal muscles by quinidine, which may result in increased plasma concentrations of digoxin (Tatro, 2011: xx). The same goes for opioids. Opioids are displaced from their binding sites by buprenorphine, and consequently the euphoric effects of opioids that commonly cause addiction are prevented (Brunton, 2012).

- **Altered transport interactions**

As discussed in the section of drug displacement interactions, P-glycoprotein is a transport protein responsible for the transport of drug molecules from the cells back into the intestinal lumen (Baxter, 2012, Hansten & Horn, 2011: PM-34, PM-35, Tatro, 2011: xviii). Potential drug interactions may also result from this.

(a) Induction of drug transport proteins

Drugs or herbal supplements such as St. John's Wort can induce P-glycoprotein and can therefore decrease drug plasma concentrations (Tatro, 2011: xviii). Table 2.12 offers a list of all the inducers and inhibitors of P-glycoprotein as adapted from Tatro (2011).

(b) Inhibition of drug transport proteins

Drugs such as amiodarone may inhibit P-glycoprotein and thus increase drug plasma concentrations (Tatro, 2011: xviii, xix). Table 2.15 summarises the inducers and inhibitors of P-glycoprotein as adapted from Tatro (2011).

Table 2.16: A list of all the inducers and inhibitors of P-glycoprotein

Inducers	Inhibitors
Rifampicin Ritonavir St. John's Wort Yohimbine	Amiodarone Atorvastatin Chlorpromazine Clarithromycin Cyclosporin Diltiazem Erythromycin Felodipine Fluphenazine Hydrocortisone Indinavir Itraconazole Ketoconazole Lidocaine Mifepristone Nelfinavir Nicardipine Nifedipine Progesterone Propranolol Quinidine Reserpine Ritonavir Saquinavir Tacrolimus Tamoxifen Testosterone Trifluoperazine Verapamil

- **Altered excretion reactions**

Drugs and their metabolites are mostly excreted in the bile or the urine. As with protein binding sites or receptor binding sites drugs can also compete for excretion at active secretion sites in the kidney. Alterations in other factors involved in excretion of drugs can influence drug excretion and thus serum concentrations of these drugs. These factors include changes in urinary pH, in active renal tubular secretion, and in renal blood flow (Baxter, 2012, Delafuente, 2003:138).

(a) Changes in urinary pH

The excretion of drugs can be altered when the pH of the urine is changed (Pleuvry, 2005:133). Drugs usually occur in an ionised or unionised form depending on the pKa value of the drug and the pH of the solution. Drugs that occur in the unionised form are lipid-soluble and can easily diffuse back into the lumen of the tubule cells. These drugs are therefore not secreted in the urine. Drugs that are ionised are lipid-insoluble and can

therefore not diffuse back into the lumen of the tubules and are then excreted in the urine (Baxter, 2012). Drugs that are weak acids will therefore be in the ionised form in an alkaline solution and will therefore be secreted in the urine. Weak basic drugs in an acid solution will be ionised and be secreted in the urine. When an overdose of a drug occurs, an interaction like this can be helpful (Baxter, 2012; Delafuente, 2003:138, 139). Aspirin and barbiturates are weak acids, and therefore increasing the pH of the urine so that it is alkaline will then increase the urinary excretion of these drugs. Sodium bicarbonate is usually used to alkalinise the urine (Corrie & Hardman, 2011:159).

(b) Changes in active tubular secretion

Drugs can compete for excretion in the renal tubules and this usually occurs at active transport systems. The most common drug that competes for excretion at the renal tubules is probenecid. Probenecid competes with weak acidic drugs such as penicillin for excretion, and as a result it prevents the excretion of penicillin and therefore increases its plasma concentrations (Pleuvry, 2005:132).

It was found that some drugs can inhibit some transporter proteins at these active transport systems (Baxter, 2012). Quinidine can inhibit excretion mediated by P-glycoprotein in the kidneys and this may increase the plasma concentration of digoxin as its excretion is inhibited (Tatro, 2011: xxv).

(c) Changes in renal blood flow

The production of prostaglandins in the kidneys causes the capillary blood vessels of the kidneys to dilate. The inhibition of the production of prostaglandins can lead to an alteration of the blood flow through the kidneys that can ultimately cause variations in the excretion of drugs through the kidneys. When this happens with drugs that have a narrow therapeutic index, it can be dangerous. NSAIDs inhibit the production of prostaglandins and can therefore cause this interaction (Baxter, 2012, Pleuvry, 2005:132, 133).

2.2.6.4 Pharmacodynamic interactions

- **Additive or synergistic interactions**

Additive interactions occur when two drugs that have the same pharmacological effect are administered together. This may lead to an augmentation of the effect. When alcohol is taken together with anxiolytics it can cause extreme drowsiness. Alcohol and anxiolytics are both central nervous system depressants and taken together, this effect is increased (Baxter, 2012; Pleuvry, 2005:130).

- **Antagonistic or opposing interactions**

Antagonistic interactions occur when two drugs with opposite pharmacological effects are co-administered. This leads to a diminished effect of the drug. Warfarin is an anticoagulant that increases the prothrombin time via the inhibition of vitamin K. When vitamin K is co-administered with warfarin it can increase the production of these clotting factors and therefore diminish the activity of warfarin (Baxter, 2012).

- **Drug or neurotransmitter uptake interactions**

Drugs can compete with each other for re-uptake back into the noradrenergic neuron. For noradrenaline to be released from the adrenergic neuron, the amphetamine derivatives need to be taken up into the adrenergic neuron. Guanethidine, which is an adrenergic neuron blocking agent, blocks the re-uptake of the amphetamines and thus the release of noradrenaline (Baxter, 2012; Pleuvry, 2005:131).

- **Disturbances in fluid and electrolyte balance**

The sensitivity of the digitalis enzyme is increased when the plasma concentrations of potassium is low. The plasma concentrations of potassium can decrease in two ways. Digitalis competes with potassium for excretion at the Na^+/K^+-ATPase pump. It therefore increases the excretion of potassium in the urine and decreases its plasma levels. Loop diuretics also compete with potassium for excretion and the same interaction occurs (Pleuvry, 2005:131).

- **Interactions at receptor sites**

Most drugs present their effects when they bind to specific receptors. Interactions can occur when agonists and antagonists that have the same affinity for a specific receptor are co-administered. An interaction like this can sometimes be beneficial. Opioid side-effects can be reversed when naloxone is administered shortly after an opioid as they react with the same receptor (Corrie & Hardman, 2011:157).

2.3 CLINICAL PROBLEM AREAS

In this section a few clinical problems concerning drug-drug interactions will be discussed. These problems include the definitions of adverse drug events and polypharmacy, as well as the significance ratings of drug reactions pertaining to warfarin use.

2.3.1 Adverse drug events

There are a few definitions to describe what adverse drug events are. The most detailed definition is given by Edwards and Aronson (2000). Their definition of an adverse drug reaction is "an appreciably harmful or unpleasant reaction, resulting from an intervention related to the use of a medical product, which predicts hazard from future administration and warrants prevention or specific treatment, or alteration of the dose regimen, or withdrawal of the product" (Edwards & Aronson, 2000). More simply it is defined as "injuries caused by drugs" (Green *et al.*, 2007:32).

There are a few examples of how adverse drug reactions present themselves. These examples include diarrhoea, constipation, nausea, hypotension, bradycardia, oedema, renal insufficiency or failure, and bleeding. Green *et al.*, (2007:32) speculate that adverse drug events cause 3.4%-7.0% of hospital admissions.

There are six types of adverse drug events. They include:

- dose-related or augmented
- non-dose-related or bizarre
- dose- and time-related or chronic
- time-related or delayed
- withdrawal or end of use
- failure of therapy or failure (Edwards & Aronson, 2000:1256)

The section of the population that is most prone to adverse drug events are the elderly. Their risk increases because they use more medications at one time because they usually have more than one chronic disease. Added to this, they also make use of more than one general practitioner (Green *et al.*, 2007:32).

2.3.2 Polypharmacy

Nobili *et al.* (2011:597) defines polypharmacy as "the use of five or more medications regardless of whether they are necessary or unnecessary". Individuals that use more than four drugs a day increase their chance for adverse drug events, medication errors, and hospital admissions (Jorgensen *et al.*, 2012:33).

As with adverse drug effects, polypharmacy can be due to patients having more than one chronic disease that ultimately leads to general practitioners prescribing more than one drug for these patients. There are several factors that increase the risk for polypharmacy. These factors include:

- age
- race or ethnicity
- gender
- level of educational achievement
- health status
- number of chronic diseases
- living arrangements
- number and characteristics of health care providers (Mizokami *et al.,* 2012:2)

2.3.3 Significance ratings of drug-drug interactions

According to Tatro (2011), not all drug interactions are equally severe. By adjusting the dose or altering the time of administration, the side-effects of these drug interactions can be avoided. There are three degrees of severity according to which drug interactions are measured. They include:

- Major: potential life-threatening events or risk of permanent damage.
- Moderate: clinical status may be deteriorated and hospital admission and therapy may be necessary.
- Minor: effects are mild; effects may bother or go unnoticed by the patient and no additional treatment is necessary (Tatro, 2011: xiv).

Drug interactions are documented by categorising it into five categories. According to Tatro (2011), these levels are:

- Established: "proven to occur in well-controlled studies."
- Probable: "very likely but not proven clinically."
- Suspected: "May occur; some good data; needs more study."
- Possible: "Could occur but data are very limited."
- Unlikely: "Doubtful; no good evidence of an altered clinical effect" (Tatro, 2011: xv).

Table 2.16 is a representation of the significance ratings assigned to a drug interaction defined by its severity and documentation level as adapted from Tatro (2011).

Table 2.17: Significance ratings of drug interactions defined by their severity and documentation level

Significance rating	Severity	Documentation level
1	Major	Suspected or less
2	Moderate	Suspected or less
3	Minor	Suspected or less
4	Major-moderate	Possible
5	Minor	Possible
	Any	Unlikely

Table 2.17 is a list of all the drugs that have an interaction with warfarin and their assigned significance rating as adapted from Tatro (2011).

Table 2.18: A list of all the drugs that potentially interact with warfarin and the assigned significance ratings of these interactions

Significance rating	1	2	4	5
Potentially interacting drug	Alteplase	Acetaminophen	Acai berry juice	Aminoglycosides
	[a]Aluminium-OH	Aminoglutethimide	Acarbose	Ascorbic acid
	Amiodarone	Argatroban	Allopurinol	Contraceptives, oral
	Amobarbital	Azathioprine	Ampicillin	Diflunisal
	Androgens (17-alkyl)	Betamethasone	Androgens (non-17-alkyl)	Disopyramide
	Antineoplastics	Bosentan	Aprepitant	Divalproex sodium
	Aspirin	Budesonide	Atazanavir	Kanamycin
	Azithromycin	Carbamazepine	Atenolol	Mineral oil
	Azole antifungals	Cefamandole	Avacado	Neomycin
	Barbiturates	Cefazolin	Bendroflumethiazide	Paromomycin
	Butabarbital	Cefoperazone	Beta-blockers	Spironolactone
	Butalbital	Cefotetan	Chamomile	Sucralfate
	Capecitabine	Cefoxitine	Chitosan	Tigecycline
	Carboplatin	Ceftriaxone	Chlorothiazide	Valproate sodium
	Celecoxib	Cephalosporins	Chlorthalidone	Valproic acid
	Cimetidine	Chloramphenicol	Cisapride	
	Clarithromycin	Cholestyramine	Citalopram	
	Cranberry juice	Ciprofloxacin	Conjugated estrogens	
	Cyclophosphamide	Corticosteroids	Cyclosporine	
	Danazol	Corticotropin	Delavirdine	
	Demeclocycline	Cortisone	Dong Quai	
	Dextrothyroxine	Cosyntropin	Duloxetine	
	Diclofenac	Danshen	Efavirenz	
	Dirithromycin	Dexamethasone	Enteral nutrition	
	Doxycycline	Dicloxacillin	Escitalopram	
	Econazole	Disulfiram	Esterified estrogens	
	Erythromycin	Ethchlorvynol	Estradiol	
	Etodolac	Ethotoin	Estrogenic substances	
	Etoposide	Fludrocortisone	Estrogens	
	Famotidine	Fosphenytoin	Estrone	
	Fenofibrate	Gefitinib	Estropipate	
	Fenoprofen	Glucagon	Ethacrynic acid	
	Fibric acid	Glutethimide	Ethanol	
	Fluconazole	Green tea	Ethinyl estradiol	
	Fluorouracil	Griseofulvin		
		Hexobarbital		

[a]*Aluminium hydroxide*

Table 2.17 (continued): A list of all the drugs that potentially interact with warfarin and the assigned significance rating of these interactions

Significance rating	1	2	4	5
Potentially interacting drug	Fluoxymesterone	Hydantoins	Felbamate	
	Flurbiprofen	Hydrocortisone	Fish oil	
	Fluvastatin	Levofloxacin	Fluoxetine	
	Gatifloxacin	Mercaptopurine	Fluvoxamine	
	Gemcitabine	Methylprednisolone	Fosamprenavir	
	Gemfibrozil	Nalidixic acid	Furosemide	
	Histamine-H_2 antagonists	Nevirapine	*Ginko biloba*	
	HMG-CoA reductase inhibitors	Norfloxacin	Ginseng	
	Ibuprofen	Ofloxacin	Glucosamine	
	Indomethacin	Penicillin G	Hydrochlorothiazide	
	Itraconazole	Penicillins	Hydroflumethiazide	
	Ketoconazole	Phenytoin	Ifosfamide	
	Ketoprofen	Piperacillin	Indapamide	
	Ketorolac	Prednisone	Indinavir	
	Levothyroxine	Quinolones	Infuenza virus vaccine	
	Liothyronine	Ranitidine	Isoniazid	
	Liotrix	Rifabutin	Isotretinoin	
	Lovastatin	Rifampicin	Leflunomide	
	Macrolide antibiotics	Rifamycins	Levonorgestrel	
	Magnesium hydroxide	Rifapentine	Loop diuretics	
	Meclofenamate	Ritonavir	Lopinavir/ritonavir	
	Mefenamic acid	Ropinirole	Mefloquine	
	Mephobarbital	Royal jelly	Menthol	
	Methimazole	Saquinavir	Mesalamine	
	Methyl Salicylate	St. John's Wort	Methyclothiazide	
	Methyltestosterone	Thiopurines	Metolazone	
	Metronidazole	Tramadol	Metoprolol	
	Miconazole	Trazodone	Mitotane	
	Minocycline	Triamcinolone	Moricizine	
	Moxifloxacin	Trovafloxacin	Myrrh	
	Nebumetone	Vitamin K	Nafcillin	
	Naproxen		Nelfinavir	
	NSAIDs		[b]NNRT inhibitors	
			Omega-3-acid ethyl esters	

[b]*Non-nucleoside reverse transcriptase inhibitors*

Table 2.17 (continued): A list of all the drugs that potentially interact with warfarin and the assigned significance rating of these interactions

Significance rating	1	2	4	5
Potentially interacting drug	[c]COX-2 selective inhibitors Oxandrolone Oxaprozin Oxymetholone Oxytetracycline Paclitaxel Pentobarbital Phenobarbital Piroxicam Primidone Propylthiouracil Quinidine Quinine Quinine derivatives Rofecoxib Rosuvastatin Salicylates Secobarbital Simvastatin Stanozolol Sulfamethizole Sulfamethoxazole Sulfasalazine Sulfinpyrazone Sulfisoxazole Sulfonamides Sulindac Telithromycin Tetracycline Thioamines Thyroid hormones Tolmetin Trimethoprim/ Sulfamethoxazole Voriconazole		Omeprazole Orlistat Oxacillin Paroxetine Polythiazide Pomegranate juice Prednisolone Progestins Propafenone Propoxyphene Propranolol Protease inhibitors Quilinggao Quinestrol Retinoids Ribavirin [d]SSRIs Sertraline Tamoxifen Terbinafine Testosterone Thiazide diuretics Ticarcillin Tolterodine Torsemide Trastuzumab Troglitazone Ubiquinone	

[c]cyclooxygenase-2 inhibitors, [d]Selective serotonin reuptake inhibitors

Hansten and Horn (2011) also developed a classification system for drug interactions. Their focus is not only on the significance of the drug interactions, but aslo on how effectively these drug interactions can be managed. Like Tatro (2011), they also have five classes of drug interactions. These classes of drug interactions are classified as follows:

Class 1: Avoid this combination. The risk of adverse patient outcome is too high for the administration of this combination of drugs.

Class 2: This combination should usually be avoided. This combination should only be administered if the benefit outweighs the risk. An alternative to one of the drugs should be used if necessary. Patient monitoring is important.

Class 3: Minimize the risk. Several alternatives are available. The drug dose and route of administration can also be changed to minimize the risk of a potential interaction. Patients should still be monitored.

Class 4: No action is necessary. The risk for adverse patient outcome is small, but there is still the possibility for interaction.

Class 5: No interaction reported. The available evidence suggests that there is no known interaction. (Hansten & Horn, 2011: xiii-xv)

Table 2.18 is a list of all the drugs that have a potential interaction with warfarin and the ratings of these interactions according to Hansten and Horn (2011).

Table 2.19: All the drugs that have a potential interaction with warfarin and the significance ratings of these drugs as adapted from Hansten & Horn (2011)

Significance rating	1	2	3	4	5
Potentially interacting drug	Phenylbutazone	Aspirin	Acetaminophen	Aminosalicylic acid	Alfuzosin
		Azapropazone	Allopurinol	Amitriptyline	Atenolol
		Bromfenac	Aminoglutethimide	Celecoxib	Azithromycin
		Cimetidine	Amiodarone	Cigarette smoke	Bumetanide
		Clofibrate	Aprepitant	Cisapride	Chlordiazepoxide
		Contraceptives, oral	Azathioprine	Cranberry juice	Conivaptan
		Danazol	Bezafibrate	Cyclosporine	Diazepam
		Dextrothyroxine	Bosentan	Diazoxide	Diphenhydramine
		Diclofenac	Capecitabine	Dicloxacillin	Dofetilide
		Diflunisal	Carbamazepine	Diltiazem	Febuxostat
		Disulfiram	Chitosan	Disopyramide	Felodipine
		Etodolac	Chloral hydrate	Ethacrynic acid	Flurazepam
		Fenoprofen	Cholestyramine	Felbamate	Furosemide
		Gemfibrozil	Ciprofloxacin	Fish oil	Grapefruit juice
		Ginkgo biloba	Clarithromycin	Garlic	Meprobamate
		Glutethimide	Colesevelam	Ginger	Moricizine
		Ibuprofen	Cyclophosphamide	Influenza vaccine	Nitrazepam
		Indomethacin	Danshen	Levamisole	Pravastatin
		Ketoprofen	Doxycycline	Levofloxacin	Psyllium
		Meclofenamate	Erythromycin	Mineral oil	Ranitidine
		Mefenamic acid	Ethanol	Omeprazole	Tacrine
		Metronidazole	Fenofibrate	Orlistat	
		Moxalactam	Fluconazole	Penicillin G	
		Nabumetone	Fluorouracil	Phenformin	
		Naproxen	Fluoxetine	Prednisone	
		Oxymetholone	Fluvoxamine	Progestins	
		Phenobarbital	Gemcitabine	Propranolol	
		Rifampicin	Ginseng	Reserpine	
		Sulfinpyrazone	Glucagon	Rofecoxib	
		Sulindac	Glyburide	Simethicone	
		Tolmetin	Griseofulvin	Simvastatin	
			Heparin	Spironolactone	
			Isoniazid	Telithromycin	
			Itraconazole		
			Ketoconazole		
			Lopinavir/Ritonavir		

Table 2.18 (continued): All the drugs that have a potential interaction with warfarin and the significance ratings of these drugs as adapted from Hansten & Horn (2011)

Significance rating	1	2	3	4	5
Potentially interacting drug			Lovastatin Mefloquine Menthol Mercaptopurine Mesalamine Miconazole Mitotane Nafcillin Nalidixic acid Neomycin Norfloxacin Ofloxacin Paroxetine Phenytoin Propafenone Propoxyphene Propylthiouracil Quilinggao Quinidine Ribavirin Sertraline St. John's Wort Sucralfate Thyroid Triclofos Trimethoprin/ Sulfamethoxazole Vitamin C Vitamin E Vitamin K Voriconazole Zafirlukast	Thiazides Ticlopidine Tigecycline Tolterodine Trazodone	

2.4 OTHER ANTICOAGULANTS

Warfarin is not the only anticoagulant used in the treatment of thrombo-embolic disorders. There is a wide range of other anticoagulant drugs that can be used for this purpose. In addition to the vitamin K antagonists, unfractioned heparin (UFH) and acetylsalicylic acid (aspirin) are also used for anticoagulation (Blommel & Blommel, 2011; Jay & Lui, 2006:30).

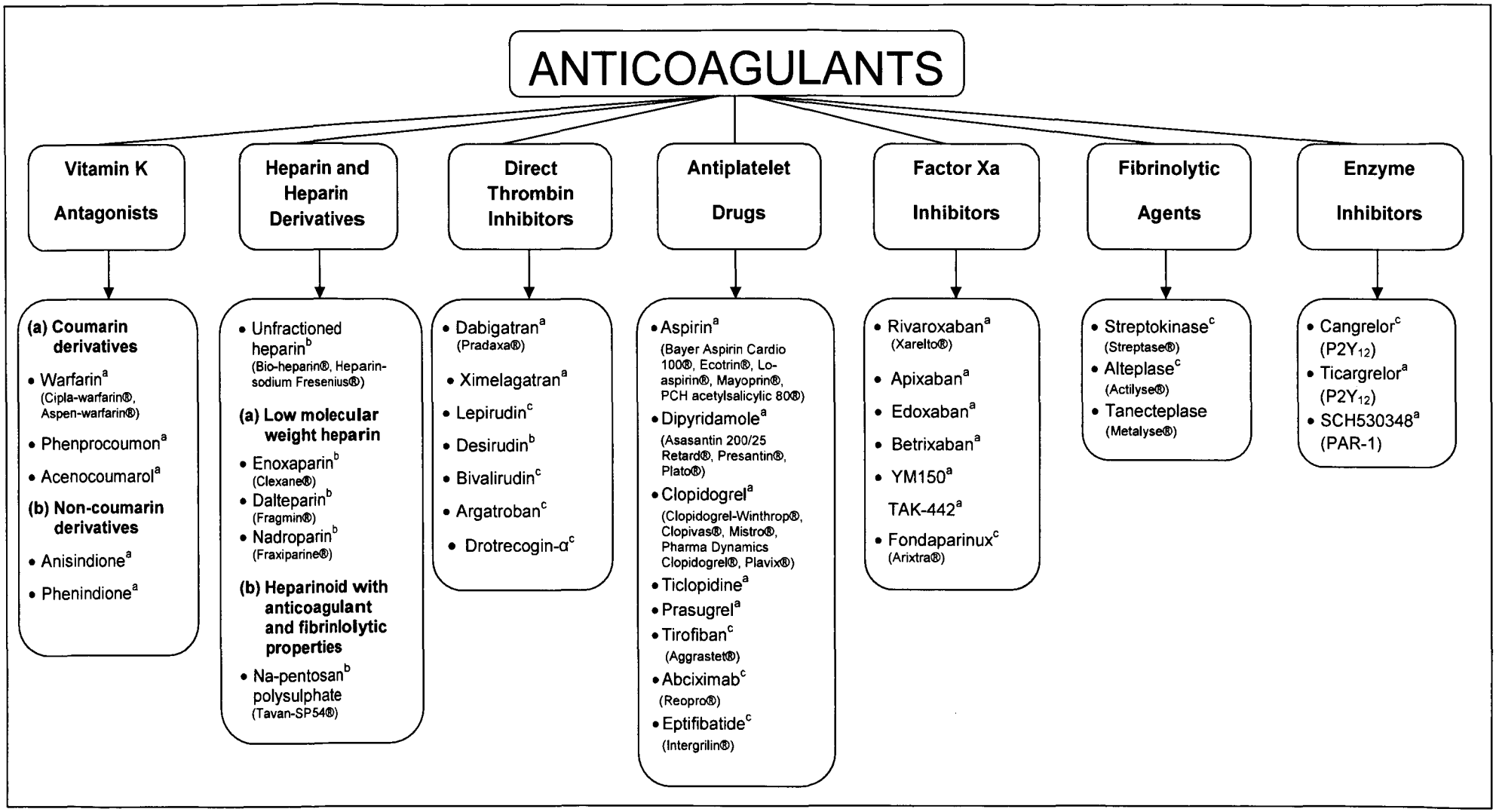
There is a significant increase in the risk of stroke in patients with atrial fibrillation. According to Lam *et al.* (2010) the risk of stroke in patients with atrial fibrillation can be prevented in about 64% of patients when treated with warfarin. However, warfarin therapy is only prescribed in 15-60% of these patients and this is due to some shortcomings with warfarin therapy. These shortcomings include:

- delayed onset/offset of pharmacological action
- narrow therapeutic index
- genetic variations in metabolism
- numerous food and drug interactions
- frequent monitoring and dose adjustments (Franchini & Mannucci, 2009:562; Lam *et al.*, 2010; Weitz, 2011:S5)

These shortcomings result in the cessation of warfarin therapy, which may increase the risk for thrombo-embolic events (Lam *et al.,* 2010).

UFH also has its share of shortcomings. These shortcomings include an intravenous route of administration, it is highly bound to plasma and endothelial proteins, laboratory monitoring is necessary, and there is a risk of heparin-induced thrombocytopenia (HIT) and osteoporosis (Franchini & Mannucci, 2009:562).

It is because of these shortcomings of current anticoagulants that the need for new, more safe and effective anticoagulants has increased (Weitz, 2011:S5). Figure 2.12 is a broad classification of current and new anticoagulants as adapted from Brunton (2012); Katzung *et al.* (2009); Snyman (2011). Each group of anticoagulants will be briefly discussed.



[a]= oral, [b]= subcutaneous, [c]= intravascular

Figure 2.12: A broad classification of current and new anticoagulants with the trade names of those anticoagulants currently on the South African market

2.4.1 VITAMIN K ANTAGONISTS

The pharmacological classification, indication, pharmacokinetics and pharmacodynamics of vitamin K antagonists have been extensively discussed in sections 2.2.2 to 2.2.5 as warfarin is a vitamin K antagonist.

There are other vitamin K antagonists available in other countries, but they are not as extensively used as warfarin (Brunton, 2012).

Phenprocoumon and acenocoumarol are used in Europe for anticoagulation. There are slight differences between these two vitamin K antagonists and warfarin. The most pronounced difference is between the half-lives of these drugs. Acenocoumarol has the shortest half-life of the three drugs (11 hours) and phenprocoumon has the longest half-life (140 hours). There are slight differences in the metabolism of these three drugs as well (Brunton, 2012; van Leeuwen *et al.*, 2008:226).

The use of phenprocoumon and acenocoumarol are sometimes preferred over warfarin. For female patients trying to get pregnant the use of acenocoumarol is preferred because of its short half-live. Treatment with phenprocoumon is not ideal because of its longer half-life. Some patients are sensitive to certain coumarins and are therefore required to change to another coumarin (van Leeuwen *et al.*, 2008).

The indandione derivatives are also still available for clinical use in some countries. The indandiones, especially phenindione, are not widely used because they are more toxic than warfarin. Some of the side-effects associated with the use of these drugs include "rashes and exfoliative dermatitis, pyrexia, diarrhoea, vomiting, sore throat, liver and kidney damage, myocarditis, agranulocytosis, leucopenia, eosinophilia, and leukaemoid syndrome" (Brunton, 2012; Sweetman, 2012).

2.4.2 HEPARIN AND HEPARIN DERIVATIVES

This group of anticoagulants consists of three groups if drugs. They include unfractioned heparin (UFH), low molecular weight heparin (LMWH) and a heparinoid with anticoagulant and fibrinolytic properties (Brunton, 2012). Fondaparinux can also be added to this group, but it will be discussed under Factor Xa inhibitors.

- **Mechanism of action**

Heparin and its derivatives all work according to the same mechanism of action (Hemker & Beguin, 1991:31). These drugs bind to a polypeptide that circulates in the body, namely antithrombin III (AT-III). AT-III is an inhibitor of the activated coagulation factors in the coagulation cascade (refer to figure 2.9). Heparin and its derivatives bind to AT-III and cause a conformational change in the binding site of AT-III where it binds to these coagulation factors. The result of this is an increase in the activity of AT-III, thus increased inhibition of the coagulation factors in the coagulation cascade (Appadu, 2010:252; Brunton, 2012; Cosmi & Palareti, 2012:388).

- **Clinical use**

Generally, all heparins are used to prevent post-operative deep vein thrombosis and to treat deep vein thrombosis (Boneu, 2000:V113). It is also used in the treatment of pulmonary embolism because it has a fast onset of action. Other uses for heparins also include angina and myocardial infarction (Brunton, 2012).

- **Side-effects**

The most common side-effects of heparin include bleeding, heparin induced thrombocytopenia (HIT), hypersensitivity reactions, alopecia, and osteoporosis after long-term exposure. Abnormalities in hepatic functions may also occur (Appadu, 2010:252; Brunton, 2012).

LMWH are a better choice over UFH as it has a more predictable dose response. It can consequently be administered in fixed doses that do not need frequent monitoring by a laboratory. The bio-availabilities of LMWH are also better than that of UFH, which means that it can be administered in low doses. Patients treated with LMWH also have a reduced risk of developing thrombocytopenia (Cosmi & Palareti, 2012:389).

Na-pentosan polysulphate is what Scully & Kakkar calls a "low-potency heparin." It is 10 times less effective than UFH (Scully & Kakkar, 1984:657).

2.4.3 DIRECT THROMBIN INHIBITORS

Dabigatran is currently the only direct thrombin inhibitor available on the South African market (Snyman, 2011). Dabigatran is administered as dabigatran etexilate, which is a pro-drug. After oral administration dabigatran etexilate is converted to dabigatran, which is the active form (Appadu, 2010:248). Dabigatran has several advantages over warfarin, including

a faster onset of action, a more predictable pharmacological profile, fewer drug and food interactions (as it is mainly excreted by the kidneys) and fewer monitoring required (Diener *et al.,* 2010:1157; Lam *et al.,* 2010).

- **Mechanism of action**

Direct thrombin inhibitors directly bind to thrombin (Factor IIa). Thrombin activates the conversion of fibrinogen to fibrin. By binding to thrombin, direct thrombin inhibitors inhibit the interaction of thrombin with fibrinogen and therefore prevent the formation of fibrin (Autar, 2009:166).

- **Clinical use**

The main clinical use for dabigatran is as thromboprophylaxis in patients with atrial fibrillation (Blommel & Blommel). Other direct thrombin inhibitors are used for several other conditions. These conditions include treatment for HIT and thromboprophylaxis in patients receiving hip replacements (Di Nisio *et al.,* 2005).

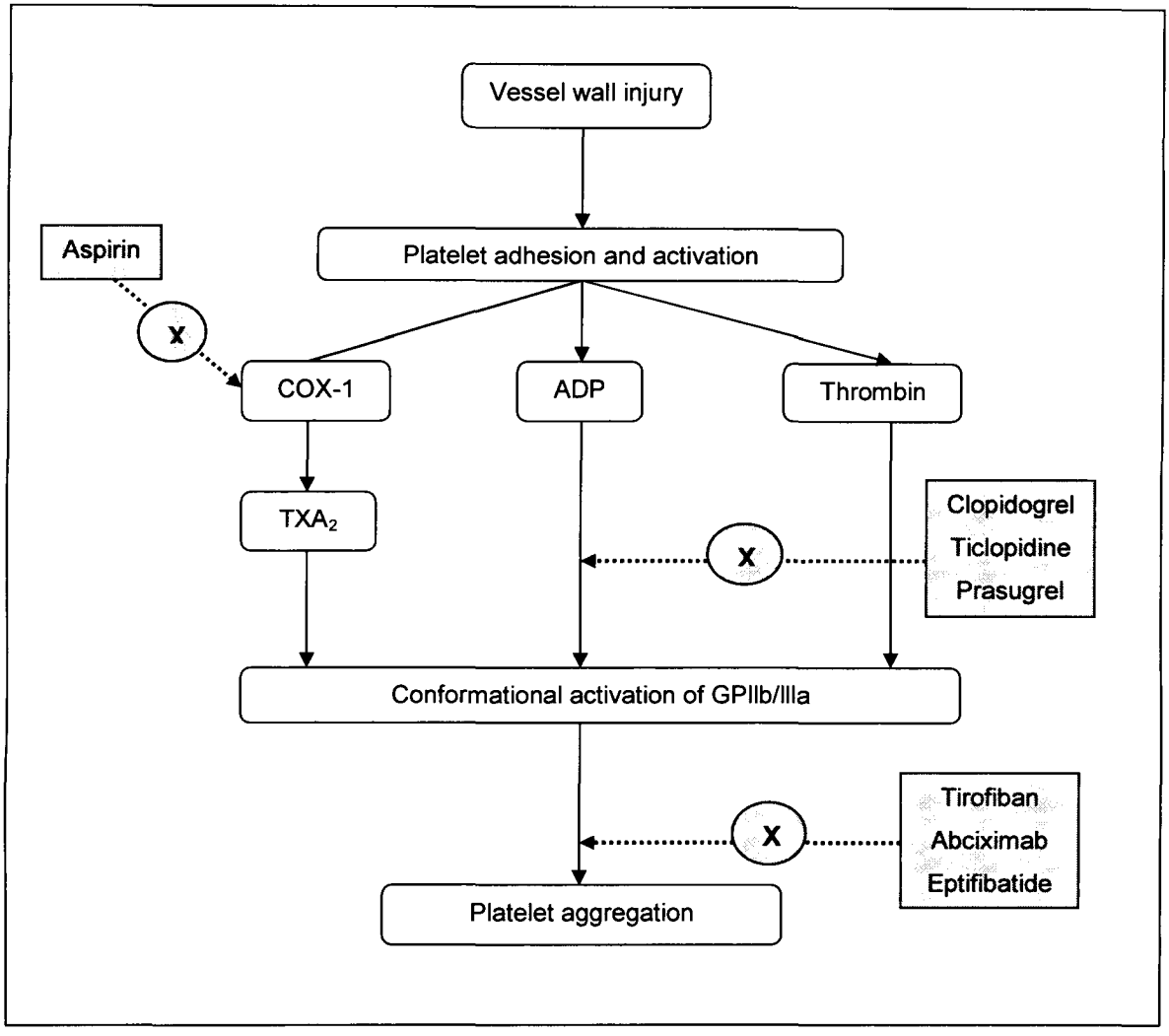
- **Side-effects**

One major risk factor associated with the use of dabigatran and other direct thrombin inhibitors is haemorrhage. Dyspepsia was also reported in about 12% of patients receiving dabigatran treatment. There have also been reports of patients that suffered myocardial infarctions while on dabigatran therapy (Blommel & Blommel, 2011).

Ximelagatran is another direct thrombin inhibitor that is under investigation. It has stable pharmacokinetics, is not metabolised by the hepatic CYP450 system and therefore has no known food and drug interactions (Halperin, 2003:432).

2.4.4 ANTIPLATELET DRUGS

To understand how antiplatelet drugs work, a quick look must be given at how platelet aggregation occurs. Figure 2.13 is a graphical representation of how platelet aggregation occurs and the sites of action of antiplatelet drugs as adapted from Brunton (2012) and Jay and Lui (2006).



COX-1 (cyclooxygenase-1), ADP (adenosine diphosphate), TXA_2 (thromboxane A_2), GPIIb/IIIa (glycoprotein IIb/IIIa)

Figure 2.13: Platelet aggregation and the sites of action of antiplatelet drugs

- **Aspirin**

Aspirin is an irreversible inhibitor of the COX-1 enzyme. COX-1 is responsible for the formation of TXA_2 which in turn induces platelet aggregation. The inhibition of COX-1 leads to a decrease in TXA_2 and therefore decreases platelet aggregation. The inhibition of platelets is permanent until new platelets are formed within 7-10 days. The antiplatelet action of aspirin is achieved at low doses of about 75 mg. Aspirin is clinically used for the treatment of acute coronary syndromes (Appadu, 2010:250; Brunton, 2012; Jay & Lui, 2006:34; Nguyen, 2011:847).

- **Dipyridamole**

Dipyridamole inhibits two enzymes namely adenosine deaminase and phosphodiesterase. The inhibition of these two enzymes leads to an increase in adenosine, adenine nucleotides and cyclic adenosine monophosphate, which ultimately hampers platelet function. Dipyridamole is also a vasodilator. Dipyridamole is used for thromboprophylaxis after surgery in conjunction with other anticoagulants (Brunton, 2012; Nguyen, 2011:850).

- **Clopidogrel**

Clopidogrel inhibits platelet aggregation by preventing ADP to bind to the $P2Y_{12}$ receptor site, which in turn causes platelet aggregation. Clopidogrel is structurally related to ticlopidine. Clopidogrel is preferred over ticlopidine because it has fewer side-effects. Clinically it is usually used as thromboprophylaxis in patients that received coronary stents. Prasugrel works similarly to clopidogrel and ticlopidine (Appadu, 2010:250; Brunton, 2012; Jay & Lui, 2006:34; Nguyen, 2011:847, 850).

- **Tirofiban**

Tirofiban is what is called a GP IIb/IIIa inhibitor. GP IIb/IIIa contains binding sites ($\alpha_{IIb}\beta_3$) for fibrinogen and von Willebrand factor. This is the final step in platelet aggregation. Tirofiban inhibits $\alpha_{IIb}\beta_3$ and therefore inhibits platelet aggregation. Abciximab and Eptifibatide works in a similar way (Brunton, 2012; Jay & Lui, 2006:34).

2.4.5 FACTOR XA INHIBITORS

Rivaroxaban is one of the first factor Xa inhibitors that have been studied in phase III clinical trials. Other newer drugs in this group that are undergoing trials are apixaban, edoxaban, betrixaban, YM150 and TAK-442 (Brunton, 2012; Lam *et al.,* 2010).

- **Mechanism of action**

The drugs in this group directly inhibit the action of factor Xa, both in the free fraction and the bound fraction (Mavrakanas & Bounameaux, 2011:48; Weitz, 2011:S7). The conversion of prothrombin or factor II to thrombin or factor IIa is therefore inhibited (refer back to figure 2.9).

- **Clinical use**

Rivaroxaban is currently being used for thromboprophylaxis in patients who received total hip and knee replacements (Autar, 2009:168; Batty & Smith, 2010:245; Lam *et al.,* 2010).

Rivaroxaban is metabolised by three CYP450 isoforms namely CYP3A4, CYP3A5 and CYP2J2. This is the reason why potent CYP450 inhibitors such as ketoconazole and ritonavir should not be administered with rivaroxaban as this can increase the plasma concentrations of this drug. Rivaroxaban is eliminated in two ways i.e, two thirds of the drug is metabolised by the liver where one third of the metabolites are excreted via the faeces and one third is excreted via the kidneys. The other route of elimination is where one third of the drug is completely excreted by the kidneys without being metabolised beforehand. Rivaroxaban can be used in combination with NSAIDs (Lam *et al.,* 2010; Mavrakanas & Bounameaux, 2011:49; Weitz, 2011:S7).

Fondaparinux is actually an antiplatelet drug that has a similar mechanism of action as UFH and LMWH. It is classified as an indirect inhibitor of factor Xa because, like the heparins, it binds to AT-III and potentiates its inhibiting effects on the coagulation factors. However, because it is a smaller molecule than UFH, it only has an effect on Factor Xa and not on thrombin. It is clinical used as thromboprophylaxis in patients receiving orthopaedic surgery (Appadu, 2010:251; Batty & Smith, 2010:245; Jay & Lui, 2006:35).

2.4.6 FIBRINOLYTIC AGENTS

Fibrinolysis is set into motion when plasminogen is converted to plasmin. Plasmin induces the degradation of fibrinogen and fibrin, hence fibrinolysis. Streptokinase binds to plasminogen and activates plasminogen to form plasmin, thus setting fibrinolysis in action. Alteplase and tanecteplase work in a similar fashion (Brunton, 2012; Katzung *et al.,* 2009:590; Weitz *et al.,* 2008:249S).

2.4.7 ENZYME INHIBITORS

Cangrelor and ticargrelor are direct inhibitors of $P2Y_{12}$ (Brunton, 2012; Weitz *et al.,* 2008:236S). SCH530348 is an inhibitor of protease-activated receptor-1 (PAR-1), which plays a role in platelet activation (Brunton, 2012).

Figure 2.14 is a graphical representation of the complete coagulation cascade and the sites of action of the anticoagulants as adapted from Brunton (2012), Jay and Lui (2006), Lam *et al.* (2010), Linder (2001), and Mavrakanas and Bounameaux (2011).

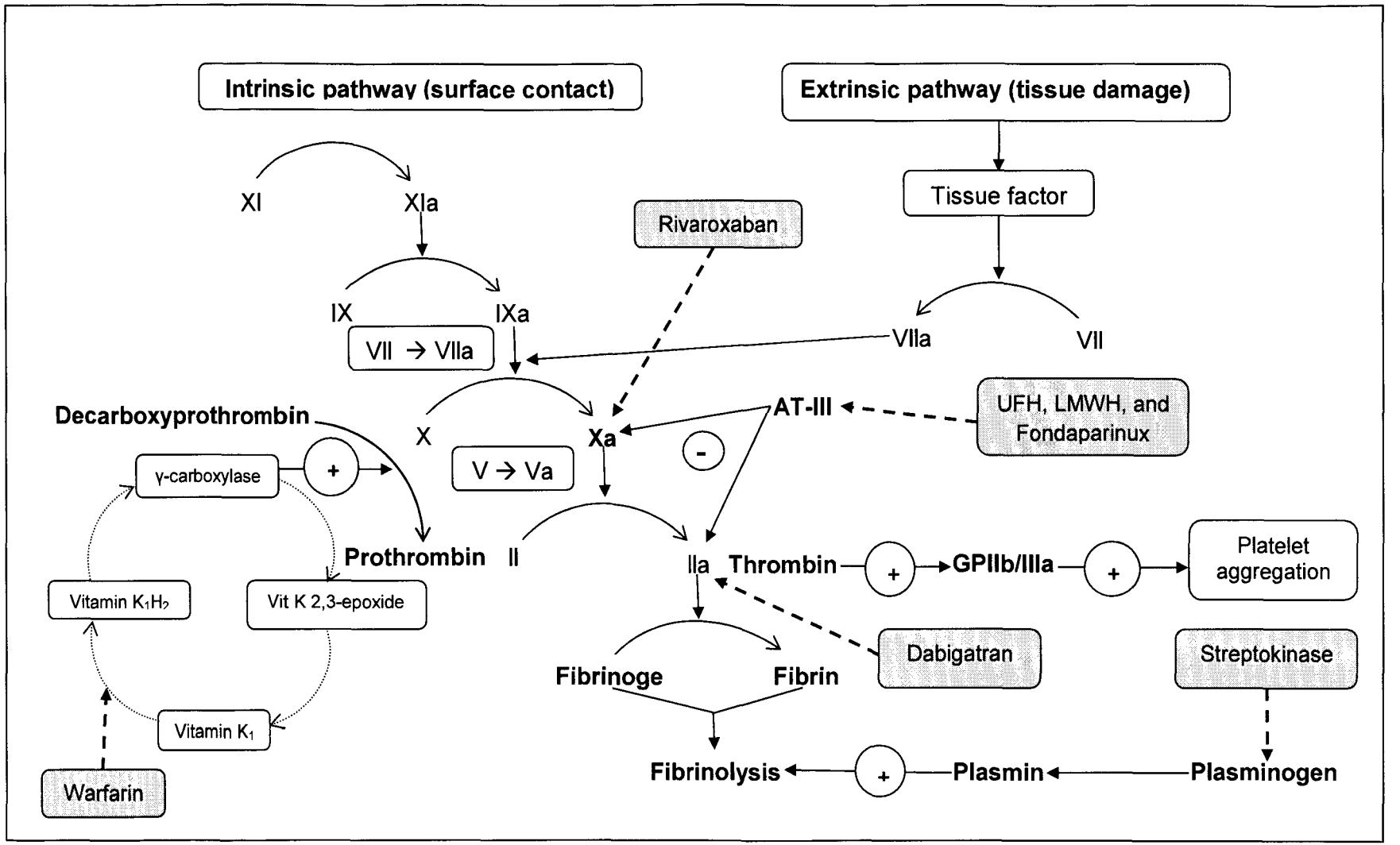


Figure 2.14: The complete coagulation cascade and the sites of action of the anticoagulants currently available on the local market

2.5 CHAPTER SUMMARY

The focus of this chapter was mainly on the pharmacokinetics and pharmacodynamics of warfarin as these are the important factors that influence the therapy of patients on warfarin. The main focus, however, is the section on clinical problem areas (section 2.3). This section focusses on the factors that complicate warfarin treatment with reference to the dosing and monitoring of warfarin therapy. The final section of the chapter (section 2.4) is an overview of other anticoagulants that are available for treatment, the classification of these drugs, and the sites of action of these drugs in the coagulation cascade. The next chapter will feature on the method of the empirical study.